

# Effects of Critical Storage Temperatures on Microbiological, Physico-chemical and Sensory Indicators of Sweetened Condensed Milk

Aleksandr Kruchinin\*<sup>id</sup>, Elena Yurova<sup>id</sup>, Ekaterina Bolshakova<sup>id</sup>, Svetlana Turovskaya<sup>id</sup>,  
Elena Illarionova<sup>id</sup>, Irina Barkovskaya<sup>id</sup>, Victoria Leonova<sup>id</sup>

All-Russian Dairy Research Institute, Lusinovskaya 35/7, 115093 Moscow, Russia;

## Abstract

**Background and Objective:** Principles of osmo and thermoanabiosis are used to produce sweetened condensed milks. Regarding their extended shelf lives, there are demands for their export to countries with various climates. However, high-positive and low-negative ambient temperatures during sweetened condensed milks transportation can affect their quality. Hence, it is important to study effects of critical storage temperatures on microbiological, physicochemical and sensory indicators of sweetened condensed milks.

**Material and Methods:** This investigation included a comprehensive study of the physicochemical, microbiological and sensory characteristics of sweetened condensed milks after storage under conditions involving multiple-stage and single-stage temperature changes within various ranges (from 5 to 50 °C; from 5 to -50 °C, from 50 to -50 °C and reverse cycles.).

**Results and Conclusion:** Analysis of samples subjected to cyclic changes, including multiple-stage heating for 9 d followed by multiple-stage cooling for 11 d, revealed that only viscosity changed relative to the control samples. In the reverse similar cycle (cooling to heating), formation of destabilized fat was observed. Moreover, changes of cycles and subsequent storage of the samples for 6 m led to increased viscosity, compared to control samples. It was established that single-stage freezing with a 14-d storage did not critically affect its quality. In contrast, rapid heating of the sweetened condensed milk up to 50 °C and storage under such critical conditions outside a cooled storage area were unacceptable. Further storage of samples subjected to cycles of single-stage freezing and heating for 6 m demonstrated a complete non-compliance with control samples for all parameters. Thus, sweetened condensed milk can be subjected to single-stage freezing to -50 °C and storage for 14 d, as well as multiple-stage cooling/freezing to -50 °C and multiple-stage heating to 50 °C following by cooling to 5 °C without loss of quality and safety during 6 m.

**Conflict of interest:** The authors declare no conflict of interest.

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### \*Corresponding author:

**Aleksandr Kruchinin \***  
All-Russian Dairy Research  
Institute, Lusinovskaya  
35/7, 115093 Moscow,  
Russia;

Tel: +7(499)236-02-36

E-mail:  
[a.kruchinin@vniimi.org](mailto:a.kruchinin@vniimi.org)

## 1. Introduction

The major challenge for the dairy industry is to ensure safety and sustainability of dairy products to meet the increasing demand of the population worldwide [1]. However, microbiological spoilage, associated biochemical problems and severe transportation temperature restrictions are great economic problems that limit supply of dairy products between countries [2]. The fundamental principle that ensures safety of dairy products and removal of foreign microflora and pathogenic microorganisms includes integrated use of pre-treatment (bactofugation, microfiltration) followed by temperature treatment (high temperature short time pasteurization, low temperature low time pasteurization, ultra-high temperature treatment, thermization,

sterilization) of raw milks [3]. To ensure products' long shelf lives, various canning approaches based on the principles of anabiosis [modifications: osmoanabiosis (sweetened condensed milk (SCM)) and xeroanabiosis (milk powder and cream) are used [4]. Moreover, [5] provides data on the possible residual presence of thermostable proteases in dried milk (especially low heat) and spore-forming bacteria, osmophilic fungi in SCM, which can lead to partial protein hydrolysis and changes in foods' sensory characteristics in case of breaking the conditions of storage and transportation. Fox et al. have verified that psychrotrophic microorganisms, seen in SCM after technological processing in storage, can cause bitterness and foreign or rancid flavor, as well as

excessive thickening of the products up to complete loss of fluidity [6]. Defects of canned milk products induced by residual microflora include negative effects on storage duration. Relatively, issues linked to the study of microbiological and biochemical spoilage mechanisms, identification of growth factors of resting forms of microorganisms and extending storage temperature ranges are important nowadays. Since SCM is one of the most storable and cost-effective dairy products, a group of researchers from Russia [7] carried out a 30-m study on the effects of addition of 0.02% w w<sup>-1</sup> (weight of fat in the product) of natural antioxidant dihydroquercetin in SCM to increase the shelf life of the product. Furthermore, researchers from China suggested addition of preservatives of 0.01% w w<sup>-1</sup> sodium dehydroacetate (E266) or 0.03% w w<sup>-1</sup> ethyl p-hydroxy-benzoate (E214), showing their effectiveness to prevent growth of osmophilic yeasts in SCM, which were the causative agents of product spoilage [8]. Routinely, SCM is popular in confectionery industries, production of various types of dairy products (e.g., ice cream and desserts) and use in daily diets [9]. Since SCM is a product of long-term storage, producing countries export it to various countries, including those with natural climates characterized by high and low temperatures. Transportation of SCM to such countries is usually carried out in refrigerated trucks with mandatory control of temperature protocols, which directly worsens the economic aspect of the export potentials. To decrease economic costs, a topical focus of research includes studying the preservative effect in SCM during storage under unregulated temperature conditions. Ryabova et al. [9,10] in an in-depth fundamental study of the storability of canned milk products reported no quality changes in SCM during storage at temperatures ranging 25 to -30 °C. However, specific values describing microbiological, physicochemical and sensory changes of SCM during freezing are not reported. Hence, the purpose of this study was to investigate effects of critical (negative and positive) storage temperatures on microbiological, physicochemical, biochemical and sensory indicators of SCM. In addition to the range of negative (up to -50 °C) and positive temperatures (up to 50 °C), a temperature difference of 100 °C (from 50 to -50 °C and vice versa) was included in the study to expand scientific and practical knowledge bases in the aspect of activation of resting forms of microorganisms. In addition, effects of single-stage and multiple-stage

heating, cooling and freezing cycle modeling controlled and uncontrolled temperature conditions during transportation were investigated. Results allow predicting and preventing defects at the early stages of formation of logistic chains of the SCM exports, as well as enabling expansion of the temperature modes of transportation without loss of quality and safety of the products, which lead to decreases in the costs of specialized transports. Expanding territories and decreasing economic costs of the export of canned dairy products, especially to low-income countries, can fully meet the global approaches to eradicate hunger and malnutrition as described by the Food and Agriculture Organization-World Health Organization (FAO-WHO).

## 2. Materials and Methods

### 2.1. Samples of sweetened condensed milk

A batch of commercially produced SCM (Promkonservy, Moscow, Russia) was purchased based on the requirements of GOST 31688-2012 canned milk, SCM and cream and specifications and technical regulations of the Customs Union TR CU 033/2013 on the safety of milk and dairy products (Table 1). The SCM was packed in tin cans with a net weight of 380 g.

### 2.2. Experimental design

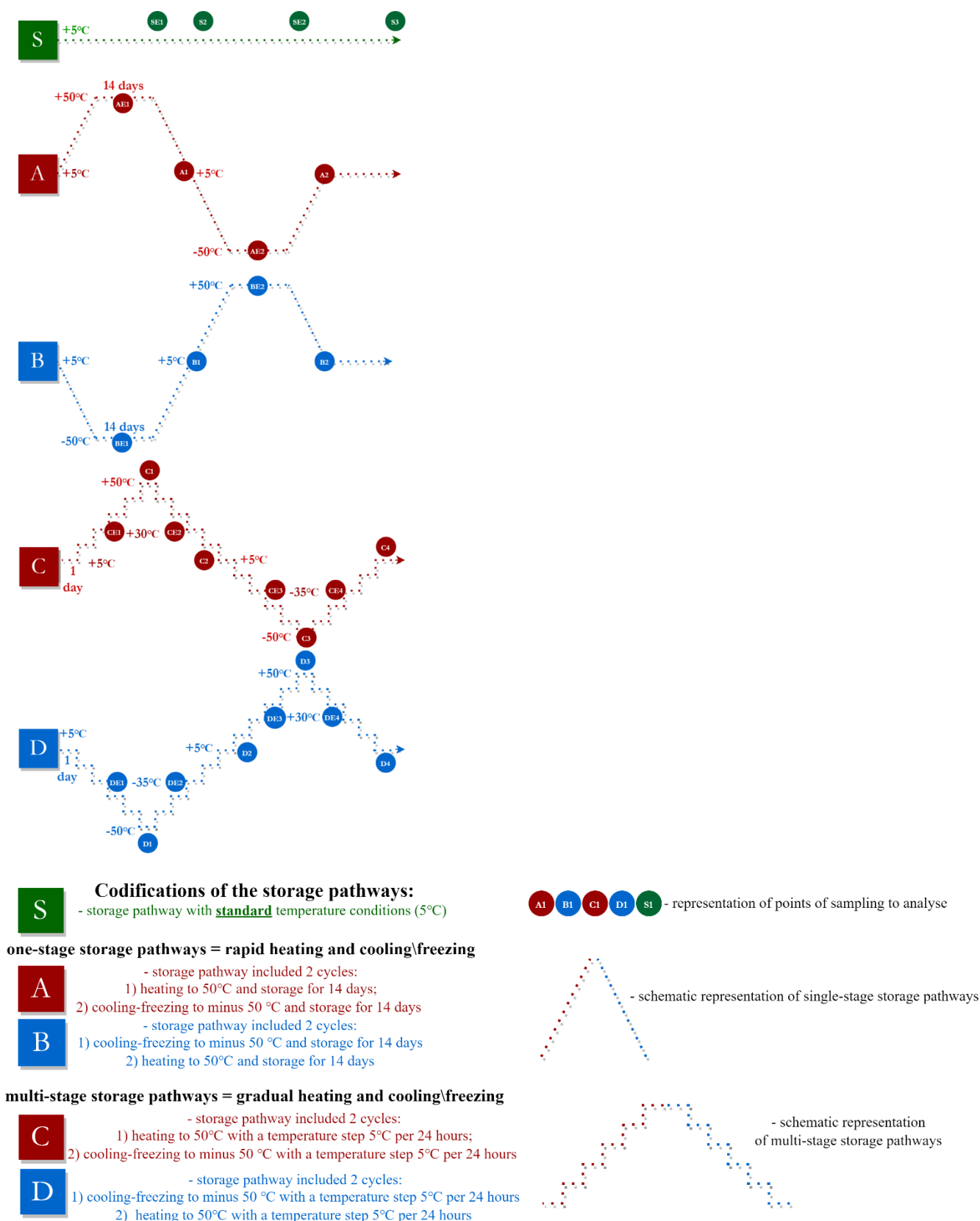
To assess effects of freezing and heating on the product quality, the following storage model consisting of paths (cycles) was designed (Figure 1). Each cycle defined as an individual storage algorithm under various types of heat exposures. Path A represented a cycle consisting of one-stage heating, storage for 14 d, one-stage cooling-freezing (50 to -50 °C), storage for 14 d and one-stage heating to a temperature of 5 °C. Path B represented the reverse cycle of Path A; for example, it began with one-stage cooling-freezing to -50 °C. Path C represented a cycle consisting of multiple-stage heating; in which, temperature increased by 5 °C every 24 h from 5 to 50 °C for 9 d, multiple-stage cooling-freezing to -50 °C with a similar temperature step (5 °C in 24 h) for 20 d and multiple-stage heating to 5 °C for 11 d. Path D included the reverse cycle of Path C, it began with multiple-stage cooling-freezing to -50 °C. Path S included the control path and storage of SCM at 5 °C. The SE1, S2, SE2 and S3 represented the sample collection points for assays.

**Table 1.** Indicators of the sweetened condensed milk based on GOST 31688-2012 and technical regulations of the Customs Union 033/2013

| Indicator                                 | Rate for SCM <sup>§</sup> | Indicator                               | Rate for SCM         |
|-------------------------------------------|---------------------------|-----------------------------------------|----------------------|
| Moisture content, %                       | ≤26.5                     | Viscosity, Pa·s                         | 3.0-15.0             |
| Sucrose content, %                        | 43.5-45.5                 | Crystals size, μm                       | ≤15.0                |
| Milk solids non-fat content, %            | ≥28.5                     | Total viable count, CFU·g <sup>-1</sup> | ≤2.0·10 <sup>4</sup> |
| Fat content, %                            | ≥8.5                      | Total coliform count in 1 g             | is not permitted     |
| Protein content of milk solids non-fat, % | ≥34.0                     | Pathogens, including salmonella in 25 g | is not permitted     |
| Titrate acidity, °T                       | ≤48.0                     |                                         |                      |

<sup>§</sup> Sweetened condensed milk





**Figure 1.** Models of single-stage and multiple-stage storages of SCM under critical temperature conditions.

Moreover, each point was a reference comparison for the acquisition points in other cycles (A, B, C and D). Thus, SE1 was the reference for AE1, BE1, C1 and D1. It is noteworthy that SE1 exactly matched C1 and D1 but differed from AE1

and BE1 in storage time by 2 d, which was an insignificant deviation in storage of the SCM control samples. The model assumed that no significant changes occurred in the control samples within 7 d. Hence, S2 corresponded to A1, B1, C2

and D2; SE2 corresponded exactly to C3 and D3 by date and with a 4-d deviation of AE2 and BE2; S3 corresponded to C4 and D4 and with a 6-d deviation of A2 and B2. The points of CE1-CE4 and DE1-DE4 were additional and entered the model to include possibility to assess the mathematical patterns of changes in the relationships. The CE1 and CE2 as well as DE3 and DE4 were investigated after being stored at 30 °C and DE1 and DE2 as well as CE3 and CE4 after being stored at -35 °C. These temperatures were chosen for additional points because at 30 °C, enhanced lactose crystallization occurred in SCM and in a range of -29 to -35 °C, the phase transition process of unbound water in SCM began [9,11].

Storage of SCM under this algorithm in the range of positive temperatures was carried out using Climostat KS-200 (SKTBU SPU, Smolensk, Russia) as well as in a range of negative temperatures using climatic chamber of SM-70/150-250 TVX (SM Klimat, St. Petersburg, Russia). Cans were set tightly to each other, simulating their real position in the box. Between the sides and top/bottom surfaces of the cans and the thermostat wall, there was a layer of corrugated cardboard, hence cans with SCM did not directly contact the heat transfer surfaces. After the SCM subjected to the described storage cycles (A, B, C, D and S), all samples were stored for 6 m.

### 2.3. Study of the quality indicators and technological characteristics of sweetened condensed milk

To study physicochemical characteristics of SCM, generally accepted and standardized methods of measurements were used. Destabilized fat content was assessed based on the principles; on which, fat content was assessed using Gerber method based on ISO 2446:2008 but without destruction of fat globule shells by sulfuric acid and with the addition of distilled water (DW) and 1 sm<sup>3</sup> of alcoholic solution of Sudan III of mass concentration 10.0 g·dm<sup>-3</sup> instead. Total nitrogen (TN) content was assessed using Kjeldahl method and Kjeltac2400 Auto Analyzer (Foss Electric, Hilleroed, Denmark) based on ISO 1871:2009, ISO 8968-1:2014. Real protein was assessed using calculation method as differences between the TN and non-protein nitrogen (NPN) with a conversion factor of 6.38. The NPN was assessed by precipitation of serum protein components with trichloroacetic acid followed by measurement of TN in the filtrate as described previously. Casein and whey proteins were analyzed via the standard method of ISO 17997-1:2004 with acid precipitation of casein and measurement of TN in the filtrate. The ratio of serum proteins to casein fractions was then calculated from the TN and NPN data. Lactose content was assessed using HPLC and liquid chromatograph of MAESTRO (INTERLAB, Moscow, Russia) based on the method of ISO 22662:2007. The pH was measured using potentiometric method and stationary pH-meter Aquasearcher AB33PH with ST 320 electrodes (Ohaus, New Jersey, USA). Freezing point (FP) was assessed in samples of SCM diluted 1:9 with water using Advanced 4250 cryoscope

(Advanced Instruments, Massachusetts, USA) based on the method of ISO 5764:2009. Titratable acidity (TA) was assessed by titrating samples with 0.1 N NaOH solution in presence of 1% alcoholic phenolphthalein indicator solution and expressed in degrees Turner (°T). Dynamic viscosity was measured at 20 °C using Brookfield DV-II+Pro rotational viscometer (Brookfield Engineering, Massachusetts, USA) with a fixed inner cylinder, chambers for samples SC4-13R(P), spindle SC4-34 at a shear rate of 20 min<sup>-1</sup> as well as Heppler viscometer VR-2110 (Ueshima Seisakusho, Tokyo, Japan). Water activity (A<sub>w</sub>) was measured at 20 °C using sorptionion-capacitance method and Hydrolab 3 device (Rotronic, Bassersdorf, Switzerland).

Contents of free amino acids (FAAs) were assessed without hydrolysis using capillary electrophoresis and KAPEL system (Lumex, St. Petersburg, Russia). Phosphate was used with the addition of beta-cyclodextrin as a background electrolyte. The FAAs were separated at 25 kV, 30 °C and 254 nm. Content of free tryptophan was assessed directly and that of other amino acids (AAs) through their phenylthiocarbamyl derivatives. The fatty acid (FA) composition was assessed using gas chromatography of Crystallux 4000M" v.2 (NPF "Meta-chrom", Yoshkar-Ola, Russia) and 5977B GC/MSD 65319-16 gas chromatography mass spectrometer (Agilent Technologies, California, USA). The browning index was assessed based on the modified method of Morales and Boekel [12], using photoelectrocolorimeter KFK-2 (Zagorsky Optical and Mechanical Plant, Sergiev Posad, Russia) at 440 and 540 nm. Enzymatic treatment to release the colored compounds was carried out using alkalase (Novozymes 2,4) and water bath (50 °C) with constant stirring for 24 h. The color change of the samples was recorded via photography using 12-MP camera of Samsung Galaxy Z Flip4 smartphone (Samsung, Suwon, South Korea) and light-tight imaging station of View gel documentation system (Helicon, Russia). Photographs were recorded with flash after 12 h of exposure at 6 °C ±2. Sample was set at equal distances from the sides of the light-tight visualization station. Qualitative assessment of the protein composition was carried out using disk-electrophoresis in polyacrylamide gel at presence of sodium dodecyl sulfate and vertical chamber Mini-PROTEAN Tetra Cell (Bio-Rad, California, USA) based on the Lammlie method. Lactose crystal and fat globule sizes were assessed in a mixture of 1 g of the sample and 9 g of glycerol using Mikmed-6 electron microscope (LOMO-Mikrosistemi, St. Petersburg, Russia), Mikro-Analiz PRO software and TCA-5.0C camera.

Degree of homogenization (HD) was analyzed in 25 ml of the sample. Sample was prepared from 40 g of condensed milk with sugar diluted with water at a ratio of 1:1.5. Then, sample was heated to 40 °C, transferred into a special pipette and centrifuged for 30 min, preserving a temperature of 40 °C. Milk was carefully drained through the lower opening of the pipette to the lower mark. The fat content was assessed





in the original sample without heating and centrifugation, as well as in the sample drained from the pipette after centrifugation. The HD was assessed by calculation via Eq. 1:

$$HD = \frac{A}{FC} \quad \text{Eq.1}$$

Where, A was fat content in the lower layer of the sample after draining from the pipette (%) and FC was fat content in the initial sample of the diluted product (%).

#### 2.4. Study of the sensory indicators of sweetened condensed milk

Sensory profile was assessed by selected tasters trained in sensory evaluation ( $n = 7$ ), monadically in separate cubicles. The group of tasters included three men and four women aged 30-45 years, who were experts from the All-Russian Research Institute of the Dairy Industry and certified in the sensory evaluation of dairy products. All the experts had at least one year or more of training in sensory evaluation expertise of dairy products. Samples were coded and analyzed on a scale from 0 to 5 points based on the developed descriptors for SCM (Table 2)

#### 2.5. Study of the microbiological indicators of sweetened condensed milk

To analyze microbiological indicators of SCM, it was sampled with a sterile dipstick after opening the tin can that was previously washed in clean warm water and wiped dry

as well as the lid was flame sterilized. A sample of SCM ( $10.0 \text{ g} \pm 0.1$ ) was stirred with a sterile spoon and added into a 200-ml sterile flask. Sample was dissolved in 90 ml of sterilized sodium chloride solution heated to  $40\text{--}45^\circ\text{C}$  and stirred for 3-5 min. Then, tenfold dilutions were prepared using a similar diluent. Dilutions were mixed thoroughly using pipettes. Suspensions were poured into Petri dishes and filled with nutrient media cooled to  $40\text{--}45^\circ\text{C}$ . To assess the total viable count and the total number of psychotrophic aerobic and facultative anaerobic microorganisms, media for total viable count (HiMedia, Mumbai, India) were used. Briefly, 0.1, 0.01 and  $0.001 \text{ cm}^3$  of the product were used for inoculation. Microorganism counting for total viable count was carried out after incubation at  $30^\circ\text{C} \pm 1$  for 72 h and counting of psychotrophic microorganisms after incubation at  $7^\circ\text{C} \pm 1$  for 7-10 d. Counting of the propagated microorganism colonies was carried out based on ISO 7218:2007. Sabouraud agar (HiMedia, Mumbai, India) with chloramphenicol was used for the identification of yeasts and molds with incubation at  $24^\circ\text{C} \pm 1$  for 3-5 d (dilutions 0.1 and 0.01). For enumeration of proteolytic bacteria, starvation agar supplemented with skim milk ( $20 \text{ g l}^{-1}$ ) was used with incubation at  $30^\circ\text{C} \pm 1$  for 48 h (dilutions 0.1 and 0.01). Proteolytic bacteria were identified by areas of lumen formed around the colonies as a result of protein degradation by proteolytic enzymes.

**Table 2.** Descriptors of the sweetened condensed milk to assess sensory indicators

| Quality indicators | 5                                                      | 4                                                                | 3                                                               | 2                                                                                                                         | 1                                                                                                                          |
|--------------------|--------------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| Color              | Uniform, white with a creamy tinge                     | Uniform, white                                                   | Uniform, distinctly creamy or white                             | Slightly non-uniform, light brown                                                                                         | Uniform, dark brown. Light yellow and dark cream spots                                                                     |
| Consistency        | Homogeneous, normally viscous                          | Homogeneous, slightly increased viscosity or decreased viscosity | Homogeneous, high viscosity, slightly sandy, excessively liquid | Heterogeneous, lactose precipitate, sandy, thickened but still flowable after stirring                                    | Heterogeneous, fat sediment, large lactose precipitate, sandy, paste-like                                                  |
| Odor               | Typical for pasteurized milk, with no extraneous odors | No extraneous odors. No pasteurization odor                      | Strong extraneous odor                                          | Odor abnormal to the product. Slight sour, yeasty and putrid odors                                                        | Abnormal odor, strong sour, yeasty, putrid, chemical and musty odors                                                       |
| Taste              | Sweet taste of pasteurized milk, no extraneous tastes  | Excessively sweet, no extraneous tastes                          | Lack of flavor, sugar syrup taste                               | Extraneous tastes: fodder, sour, yeasty, faint rancid, greasy, caramelized, metallic and others, not clearly identifiable | Strong extraneous tastes: fodder, salty, rotten, musty, rancid, caramelized, metallic and others, not clearly identifiable |

The quality index "Overall Impression" was calculated as the arithmetic mean of taste, odor, color and consistency.

For identification of lipolytic microorganisms, media for total viable count were used as a base, then sterile melted beef fat was poured onto the bottom of a Petri dish until a uniform thin layer was formed. After solidification of the fat layer, tested isolates were inoculated into the dishes. Then, the media was poured into Petri dishes. Incubation carried out at  $20\text{--}23^\circ\text{C}$  for 5-6 d (dilutions 0.1 and 0.01). Colonies of lipolytic bacteria that decomposed fat formed on the media lumen zones. Total coliform count was carried out using

liquid Kessler media (Chimmed, Moscow, Russia). This method was based on the ability of coliforms to digest lactose in the media with the formation of gas and acid at  $37^\circ\text{C} \pm 1$  for 24 h. Sign of coliform propagation on liquid Kessler media included visually observed gas accumulation in the float. To assess the total coliform count, 1.0, 0.1 and 0.01 ml of the product were inoculated in 5 ml of nutrient media. Identification of *Salmonella* spp. was carried out based on ISO 6785:2001. Then, 25 g of the product were added to a

500-ml sterile flask and 225 ml of sterile buffered peptone water were poured into the flask and stirred thoroughly. The resulting suspension was incubated at 37 °C for 24 h. Then, 0.1 ml of the culture was transferred into 10 ml of RVS media. Tubes with media were incubated at 41.5 °C for 24 h. Cultures on RVS broth were transferred to Hektoen enteric agar (HEA) using bacteriological loops and incubated at 37 °C for 24 h. *Listeria monocytogenes* was identified using *Listeria* selective agar base (LSAB) added with selective supplement and incubated at 37 °C for 24 h.

## 2.6. Statistics

All statistical analyses were carried out using R statistical software package v.4.1.1 in RStudio program (Posit Software, Massachusetts, USA). Tukey's method was used to assess statistical significance and pairwise mean comparisons after an ANOVA for the data set. All parameters were analyzed in triplicate.

## 3. Results and Discussion

### 3.1. Physicochemical characteristics of the sweetened condensed milk

Changes in  $A_w$  of the SCM system during storage are usually associated with significant fluctuations in carbohydrate (inversion of sugars) and mineral compositions. Based on the results, no significant changes were seen in  $A_w$  in samples subjected to one-stage and multiple-stage temperature exposures, possibly due to the absence of significant effects of the biochemical processes on changes in bound water content (Table 3).

Analysis of acidity values [titratable (TA) and pH] of the control samples of SCM showed no changes during storage at 5 °C. In one-stage heating-cooling cycle (Path A), a linear dependence of TA increase and pH decrease on temperature effects was observed at each cycle stage. After a heating-cooling cycle of 14 d (A1), TA increased by 14 °T and pH decreased by 0.21, relative to the S point, possibly as a consequence of the partial denaturation of whey proteins, changing dissociation equilibrium between the true and colloid-soluble phases. A further negative trend in acidity was seen during the subsequent freezing, resulting in a TA at the endpoint (A2) of 69 °T and a pH of 5.99. The initial freezing itself (Path B) did not result in changes in acidity (B1). Subsequent heating (B2) intensified the process by significantly increased titratable acidity by 23°T and decreased pH by 0.43, which might indicate the primary role of positive critical temperatures on the course of biochemical reactions associated with the polypeptide complex. Pre-freezing before heating increased rates of the highlighted processes. Multiple-stage heating and freezing did not lead to changes in acidity, compared to the control samples. Thus, gradual temperature changes when reaching maximum positive and negative values with no exposure include no effects on the titratable and pH of the samples. The data on changes in acidity correlate with the results achieved

previously by researchers in [13], who designed a model for assessing storability of the condensed milk by changes in absorption, hydroxymethylfurfural content and ability to bleach coffee. Changes in viscosity of SCM were resulted from structural transformations of the internal components (protein, fat, carbohydrates and water). Transformation of the protein not only affected increased acidity, but also affected the dynamic viscosity. Analysis results of the sample dynamic viscosity subjected to one-stage cycles of thermal exposure showed significant increases in its value regardless of the sequence of heating and cooling at an average of six times, regarding values at the initial point. Values of dynamic viscosity in single-stage cycles were averagely higher by a factor of 3 at the end point of the cyclic experiment, compared to multiple-stage cycles. According to [14], heat treatment (temperature and exposure) is able to modify the rheological characteristics of SCM. Moreover, size and shape of the casein clusters and their interactions with the carbohydrates of SCM can affect changes in viscosity, explaining thermal effects of Paths A and B. It is noteworthy that despite the fact that SCM does not contain polysaccharides that is suggested [15] to prevent crystallization of lactose at low temperatures, high viscosity of SCM because of the high content of solids did not allow development of the defect during freezing.

### 3.2. Changes in the carbohydrate fraction of sweetened condensed milk

In this study, changes in the carbohydrate fraction of SCM under effects of critical positive and negative storage temperatures were investigated. Investigation was carried out by changes in the lactose content and size of its crystals as well as freezing point and browning index (Table 4).

Decreases of freezing point in A2, B2 and D4 samples were linked to hydrolysis of sucrose, as it was more susceptible to hydrolysis to monosaccharides than lactose. Lactose content after all thermal effects did not correlate with the changes in freezing points. A clear dependence of the freezing point was reported towards lowering after holding the samples at positive critical temperatures in the process of one-stage thermal exposure, as well as multiple-stage exposure, in the cycle of freezing-heating. Increases in crystal sizes were seen for the samples of Path A, which was possibly linked to the dissolution of nominally standardized crystals and occurrence of recrystallization after cooling.

For the samples of Path B in cooling-heating cycle after cooling, no increases in crystal size were detected. However, positive changes in crystal size were observed in a similar cycle after heating and subsequent cooling. No significant changes in crystal sizes were recorded in the samples of Paths C and D, indicating that the multiple-stage mode of cooling and heating was gentler for the effects on the carbohydrate components, compared to Paths A and B.

**Table 3.** Physicochemical characteristics of the sweetened condensed milk stored under various temperature conditions



| Cycle type     | Indicators               |                           |                          |                           |                              |
|----------------|--------------------------|---------------------------|--------------------------|---------------------------|------------------------------|
| Name of sample | Water activity           | Titrateable acidity, (°T) | pH                       | Heppler Viscosity, (Pa·s) | Brookfield Viscosity, (Pa·s) |
| S              | 0.848±0.002 <sup>a</sup> | 41±2 <sup>e</sup>         | 6.36±0.03 <sup>a</sup>   | 8.4±0.2 <sup>o</sup>      | 8.9±1.1 <sup>f</sup>         |
| SE1            | 0.850±0.001 <sup>a</sup> | 39±3 <sup>e</sup>         | 6.37±0.08 <sup>a</sup>   | 12.8±0.7 <sup>jkl</sup>   | 12.8±2.0 <sup>ef</sup>       |
| S2             | 0.856±0.005 <sup>a</sup> | 39±3 <sup>e</sup>         | 6.38±0.06 <sup>a</sup>   | 12.6±0.3 <sup>klmn</sup>  | 14.4±1.5 <sup>e</sup>        |
| SE2            | 0.852±0.006 <sup>a</sup> | 41±2 <sup>e</sup>         | 6.40±0.10 <sup>a</sup>   | 11.3±0.5 <sup>lmn</sup>   | 13.7±1.2 <sup>e</sup>        |
| S3             | 0.849±0.003 <sup>a</sup> | 40±3 <sup>e</sup>         | 6.40±0.10 <sup>a</sup>   | 12.0±0.4 <sup>klmn</sup>  | 13.9±1.0 <sup>e</sup>        |
| AE1            | 0.852±0.002 <sup>a</sup> | 48±2 <sup>d</sup>         | 6.23±0.04 <sup>ab</sup>  | 21.4±0.6 <sup>d</sup>     | 25.6±2.1 <sup>bc</sup>       |
| A1             | 0.852±0.004 <sup>a</sup> | 55±2 <sup>c</sup>         | 6.15±0.10 <sup>bc</sup>  | 25.6±0.3 <sup>c</sup>     | 29.2±2.0 <sup>b</sup>        |
| AE2            | 0.849±0.001 <sup>a</sup> | 58±1 <sup>bc</sup>        | 6.01±0.02 <sup>cd</sup>  | 29.2±0.3 <sup>b</sup>     | 26.1±2.3 <sup>bc</sup>       |
| A2             | 0.853±0.003 <sup>a</sup> | 69±2 <sup>a</sup>         | 5.99±0.06 <sup>cde</sup> | 53.4±0.5 <sup>a</sup>     | >64.0 <sup>a</sup>           |
| BE1            | 0.853±0.004 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.39±0.10 <sup>a</sup>   | 13.7±0.2 <sup>ij</sup>    | 13.1±1.5 <sup>ef</sup>       |
| B1             | 0.852±0.002 <sup>a</sup> | 41±1 <sup>ab</sup>        | 6.38±0.14 <sup>a</sup>   | 12.0±0.4 <sup>klmn</sup>  | 12.2±2.1 <sup>ef</sup>       |
| BE2            | 0.849±0.001 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.26±0.10 <sup>ab</sup>  | 20.6±0.4 <sup>de</sup>    | 27.7±1.4 <sup>b</sup>        |
| B2             | 0.850±0.004 <sup>a</sup> | 64±2 <sup>ab</sup>        | 5.93±0.09 <sup>de</sup>  | 53.1±0.7 <sup>a</sup>     | >64.0 <sup>a</sup>           |
| CE1            | 0.850±0.005 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.40±0.05 <sup>ab</sup>  | 11.2±0.1 <sup>mn</sup>    | 13.0±1.0 <sup>ef</sup>       |
| C1             | 0.848±0.006 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.35±0.01 <sup>a</sup>   | 13.3±0.2 <sup>ijk</sup>   | 19.9±1.0 <sup>d</sup>        |
| CE2            | 0.852±0.001 <sup>a</sup> | 44±1 <sup>de</sup>        | 6.36±0.07 <sup>a</sup>   | 16.6±0.3 <sup>fg</sup>    | 21.9±1.6 <sup>cd</sup>       |
| C2             | 0.853±0.004 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.36±0.01 <sup>a</sup>   | 17.9±0.7 <sup>f</sup>     | 22.9±2.0 <sup>cd</sup>       |
| CE3            | 0.852±0.004 <sup>a</sup> | 44±1 <sup>de</sup>        | 6.36±0.01 <sup>a</sup>   | 16.6±0.4 <sup>fg</sup>    | 21.9±1.0 <sup>cd</sup>       |
| C3             | 0.851±0.002 <sup>a</sup> | 43±3 <sup>de</sup>        | 6.36±0.03 <sup>a</sup>   | 16.0±0.3 <sup>gh</sup>    | 20.9±1.4 <sup>d</sup>        |
| CE4            | 0.849±0.001 <sup>a</sup> | 43±2 <sup>de</sup>        | 6.33±0.03 <sup>ab</sup>  | 15.6±0.2 <sup>gh</sup>    | 19.2±1.0 <sup>d</sup>        |
| C4             | 0.849±0.003 <sup>a</sup> | 41±2 <sup>e</sup>         | 6.35±0.01 <sup>ab</sup>  | 14.7±0.2 <sup>hi</sup>    | 22.2±1.9 <sup>cd</sup>       |
| DE1            | 0.848±0.002 <sup>a</sup> | 42±2 <sup>de</sup>        | 6.39±0.02 <sup>a</sup>   | 10.6±0.6 <sup>mn</sup>    | 12.1±1.0 <sup>ef</sup>       |
| D1             | 0.850±0.004 <sup>a</sup> | 42±1 <sup>de</sup>        | 6.38±0.06 <sup>a</sup>   | 11.3±0.7 <sup>lmn</sup>   | 13.6±1.1 <sup>e</sup>        |
| DE2            | 0.854±0.004 <sup>a</sup> | 43±1 <sup>de</sup>        | 6.39±0.06 <sup>a</sup>   | 14.5±0.8 <sup>hi</sup>    | 14.3±1.1 <sup>e</sup>        |
| D2             | 0.854±0.002 <sup>a</sup> | 44±3 <sup>de</sup>        | 6.38±0.01 <sup>a</sup>   | 11.2±0.3 <sup>mn</sup>    | 12.7±1.0 <sup>ef</sup>       |
| DE3            | 0.850±0.003 <sup>a</sup> | 41±1 <sup>e</sup>         | 6.40±0.02 <sup>a</sup>   | 12.7±0.1 <sup>klmn</sup>  | 13.8±1.2 <sup>e</sup>        |
| D3             | 0.850±0.001 <sup>a</sup> | 41±3 <sup>e</sup>         | 6.34±0.04 <sup>ab</sup>  | 16.6±0.7 <sup>fg</sup>    | 20.9±1.4 <sup>d</sup>        |
| DE4            | 0.849±0.001 <sup>a</sup> | 43±1 <sup>de</sup>        | 6.33±0.01 <sup>ab</sup>  | 16.5±0.9 <sup>fg</sup>    | 19.9±1.5 <sup>d</sup>        |
| D4             | 0.849±0.001 <sup>a</sup> | 42±1 <sup>de</sup>        | 6.36±0.06 <sup>a</sup>   | 19.7±0.8 <sup>e</sup>     | 22.9±1.6 <sup>cd</sup>       |

<sup>a-o</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA;  $p < 0.05$ .

Moreover, all cyclic storage paths included negative effects on the average crystal size, which increased compared to the controls ( $3.84 \mu\text{m} \pm 0.28$ ) and was in a range of  $4.47 \pm 0.47$  to  $5.21 \pm 0.16 \mu\text{m}$  by the end of the experiment. However, this did not exceed the standardized values of the parameter ( $15 \mu\text{m}$ ). It is noteworthy that the formation of lactose crystals larger than  $15 \mu\text{m}$ , reported in all paths at various stages, did not affect the sensory indicators as the average crystal size was less than  $5 \mu\text{m}$ . It has been reported that lactose crystals of less than  $10 \mu\text{m}$  do not cause defects such as sandiness and powderiness [4,16,17]. Mehta has suggested that not only the size but also the number of crystals with large sizes are important in the formation of sensory defects in SCM [18]. Analysis of the browning index revealed effects on the Maillard reaction intensification by the cooling cycle to critical temperatures after preheating in one-stage storage cycle (Path A). In this cycle, increases of the index for the samples from the beginning of the experiment after heating (A1) alone in relationship to S was  $0.279 \text{ OD/g}$  and after cooling (A2) in relationship to A1 was  $0.529 \text{ OD/g}$ , which is almost twice the value achieved for the first period. In the samples of Path B, no increases in the index were seen after the cooling. In contrast, the colored

compound index increased by  $0.589 \text{ OD.g}^{-1}$  after the subsequent heating, which was almost two times higher than that achieved for a single heating period (Path A). This indicated that pre-freezing before heating in the single-stage storage cycle as well as freezing following heating intensified development of Maillard reaction. Assessment of the changes in the index of Paths C and D samples showed non-significant results, ranging  $0.046\text{--}0.049 \text{ OD/g}$ .

### 3.3. Changes in the protein fraction of sweetened condensed milk

During storage and transportation of SCM, especially under the effects of critical positive and negative temperatures, protein fraction of the milk system may be subjected to significant transformation. To study stability /instability of the protein system, indicators of changes in protein and NPN (Table 5), molecular-mass distribution of protein fractions (Fig. 2) and content of FAAs were investigated (Fig. 3). Analysis of data in Table 5 shows statistically non-significant changes in the values of NP and NPN, which were within the limits of measurement accuracy of the method.

**Table 4.** Changes in indicators characterizing carbohydrate fraction of the sweetened condensed milk stored under various temperature conditions

| Cycle type     | Indicators             |                               |                              |                             |                                       |
|----------------|------------------------|-------------------------------|------------------------------|-----------------------------|---------------------------------------|
| Name of sample | Lactose, (%)           | Freezing point, °C            | Average crystals size, (μm)  | Maximum crystals size, (μm) | Browning index, (OD g <sup>-1</sup> ) |
| S              | 12.3±0.1 <sup>ab</sup> | - 0.415±0.001 <sup>d</sup>    | 3.50±0.34 <sup>efgh</sup>    | 8.70±1.17 <sup>e</sup>      | 0.066±0.001 <sup>i</sup>              |
| SE1            | 12.9±0.2 <sup>a</sup>  | - 0.414±0.001 <sup>de</sup>   | 3.80±0.42 <sup>bcdefgh</sup> | 7.99±2.07 <sup>e</sup>      | 0.041±0.001 <sup>k</sup>              |
| S2             | 12.8±0.4 <sup>a</sup>  | - 0.415±0.001 <sup>d</sup>    | 3.39±0.35 <sup>gh</sup>      | 11.05±2.65 <sup>cde</sup>   | 0.054±0.002 <sup>j</sup>              |
| SE2            | 12.5±0.2 <sup>ab</sup> | - 0.411±0.003 <sup>def</sup>  | 3.02±0.10 <sup>h</sup>       | 12.87±2.14 <sup>bcde</sup>  | 0.064±0.002 <sup>i</sup>              |
| S3             | 12.3±0.3 <sup>ab</sup> | - 0.411±0.001 <sup>def</sup>  | 3.84±0.28 <sup>bcdefgh</sup> | 11.60±1.10 <sup>cde</sup>   | 0.044±0.003 <sup>k</sup>              |
| AE1            | 12.8±0.2 <sup>a</sup>  | - 0.434±0.003 <sup>ab</sup>   | 4.32±0.37 <sup>abcde</sup>   | 18.55±2.39 <sup>ab</sup>    | 0.184±0.001 <sup>f</sup>              |
| A1             | 12.9±0.3 <sup>a</sup>  | - 0.431±0.003 <sup>b</sup>    | 3.99±0.28 <sup>bcdefg</sup>  | 8.89±2.44 <sup>e</sup>      | 0.345±0.001 <sup>d</sup>              |
| AE2            | 12.5±0.2 <sup>ab</sup> | - 0.430±0.003 <sup>b</sup>    | 4.48±0.11 <sup>abcd</sup>    | 12.48±2.47 <sup>bcde</sup>  | 0.507±0.002 <sup>c</sup>              |
| A2             | 12.3±0.3 <sup>ab</sup> | - 0.439±0.001 <sup>a</sup>    | 5.21±0.16 <sup>a</sup>       | 11.89±1.54 <sup>cde</sup>   | 0.874± 0.004 <sup>a</sup>             |
| BE1            | 12.6±0.4 <sup>a</sup>  | - 0.413±0.002 <sup>de</sup>   | 3.79±0.13 <sup>bcdefgh</sup> | 15.70±1.91 <sup>abcd</sup>  | 0.073±0.001 <sup>h</sup>              |
| B1             | 13.0±0.3 <sup>a</sup>  | - 0.409±0.001 <sup>efgh</sup> | 3.70±0.29 <sup>cdefgh</sup>  | 10.77±2.47 <sup>cde</sup>   | 0.052±0.001 <sup>j</sup>              |
| BE2            | 12.5±0.4 <sup>ab</sup> | - 0.406±0.001 <sup>fgh</sup>  | 3.91±0.41 <sup>bcdefgh</sup> | 12.63±1.53 <sup>bcde</sup>  | 0.236±0.003 <sup>e</sup>              |
| B2             | 11.8±0.4 <sup>b</sup>  | - 0.432±0.001 <sup>b</sup>    | 4.66±0.27 <sup>ab</sup>      | 13.03±2.88 <sup>bcde</sup>  | 0.641±0.003 <sup>b</sup>              |
| C1             | 12.4±0.2 <sup>ab</sup> | - 0.405±0.001 <sup>gh</sup>   | 4.31±0.43 <sup>abcdef</sup>  | 21.43±1.78 <sup>a</sup>     | 0.110±0.001 <sup>g</sup>              |
| C2             | 12.3±0.2 <sup>ab</sup> | - 0.404±0.003 <sup>h</sup>    | 4.03±0.18 <sup>bcdefg</sup>  | 7.39±1.33 <sup>e</sup>      | 0.110±0.001 <sup>g</sup>              |
| C3             | 12.5±0.3 <sup>ab</sup> | - 0.410±0.002 <sup>defg</sup> | 3.84±0.15 <sup>bcdefgh</sup> | 8.08±1.26 <sup>e</sup>      | 0.113±0.001 <sup>g</sup>              |
| C4             | 12.6±0.2 <sup>ab</sup> | - 0.405±0.001 <sup>gh</sup>   | 4.47±0.47 <sup>abcd</sup>    | 16.38±1.88 <sup>abc</sup>   | 0.112±0.002 <sup>g</sup>              |
| D1             | 12.5±0.4 <sup>ab</sup> | - 0.409±0.002 <sup>efgh</sup> | 3.40±0.16 <sup>fgh</sup>     | 8.77±2.52 <sup>e</sup>      | 0.111±0.002 <sup>g</sup>              |
| D2             | 11.8±0.1 <sup>b</sup>  | - 0.411±0.002 <sup>def</sup>  | 3.57±0.31 <sup>defgh</sup>   | 10.06±1.67 <sup>de</sup>    | 0.111±0.001 <sup>g</sup>              |
| D3             | 12.6±0.2 <sup>ab</sup> | - 0.423±0.002 <sup>c</sup>    | 4.55±0.11 <sup>abc</sup>     | 13.05±2.04 <sup>bcde</sup>  | 0.114±0.002 <sup>g</sup>              |
| D4             | 12.4±0.3 <sup>ab</sup> | - 0.431±0.001 <sup>b</sup>    | 4.61±0.37 <sup>abc</sup>     | 18.57±1.42 <sup>ab</sup>    | 0.115±0.001 <sup>g</sup>              |

<sup>a-k</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA;  $p < 0.05$ .

Results of the electrophoretic separation of the samples at the end of Path B showed decreases in the content of low-molecular-weight (LMW) fractions, particularly  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, with simultaneous increases in high-molecular-weight (HMW) protein molecules (Fig. 2). These results were similar to those of Curlej et al., who detected decreases in whey proteins ( $\alpha$ -la and  $\beta$ -lg) in a milk system exposed to high temperatures of electrophoretic analysis [19]. Analysis of the samples of Path A indicated that heat exposure at critical positive (A1) and critical negative (A2) temperatures stimulated protein changes. No significant changes in qualitative protein composition were seen for samples of Paths C and D. This could be explained by the fact that acidity (a denaturing factor) did not change significantly under multiple-stage heat exposures. In the experiment, time of heat exposure for Paths A and B samples was significantly higher than that of Paths C and D (14 times at 50 °C), verifying the hypotheses of [15], who underlined significant roles of the temperature and exposure in protein changes. Moreover, changes in the FAA content were analyzed (Fig. 3).

Thus, qualitative differences in the content of FAAs were demonstrated in the samples that were subjected to storage paths of the single-stage cycle model (A and B) and the multiple-stage cycle model (C and D). In heating-cooling and cooling-heating cycles of the single-stage storage, changes in the quantities of nine AAs out of 20 AAs were reported. Free forms of other AAs were not observed. In cycles of multiple-stage temperature exposure, five further AAs (glutamic acid and glutamine, asparagic acid and asparagine and alanine)

were subjected to change in addition to the nine highlighted ones; in which, AAs were addressed as quite active in reactions of glycation.

### 3.4. Changes in the lipid fraction of sweetened condensed milk

In the process of storage and transportation of SCM under the effects of critical positive and negative temperature, lipid fraction might include changes. The major reference points of the studies characterizing the changes included indicators of the content of destabilized fat, sizes of fat globules (Table 6) and compositions of FAs (Fig. 4). Analysis of the lipid fraction of SCM samples revealed that only the multiple-stage cooling provided in Paths C and D facilitated release of trace quantities of destabilized fats. This might be due to mechanical damages to the fat globules during the multiple-stage cooling-freezing process. Release of destabilized fat might be associated with the process of migratory recrystallization, which was most often stimulated by fluctuations in storage temperature [20], which was in Path D because of multiple-stage freezing process. Large ice crystals formed at the expense of small ones are mechanical threats to the membranes of fat globules [21,22]. In the cooling-heating cycle of the multiple-stage storage cycle (Path D) after a period of freezing to critical negative temperatures followed by heating to 5 °C, releases of destabilized fat were seen in the samples, the content of which persisted in the samples of this path until the end of the cycling experiment.



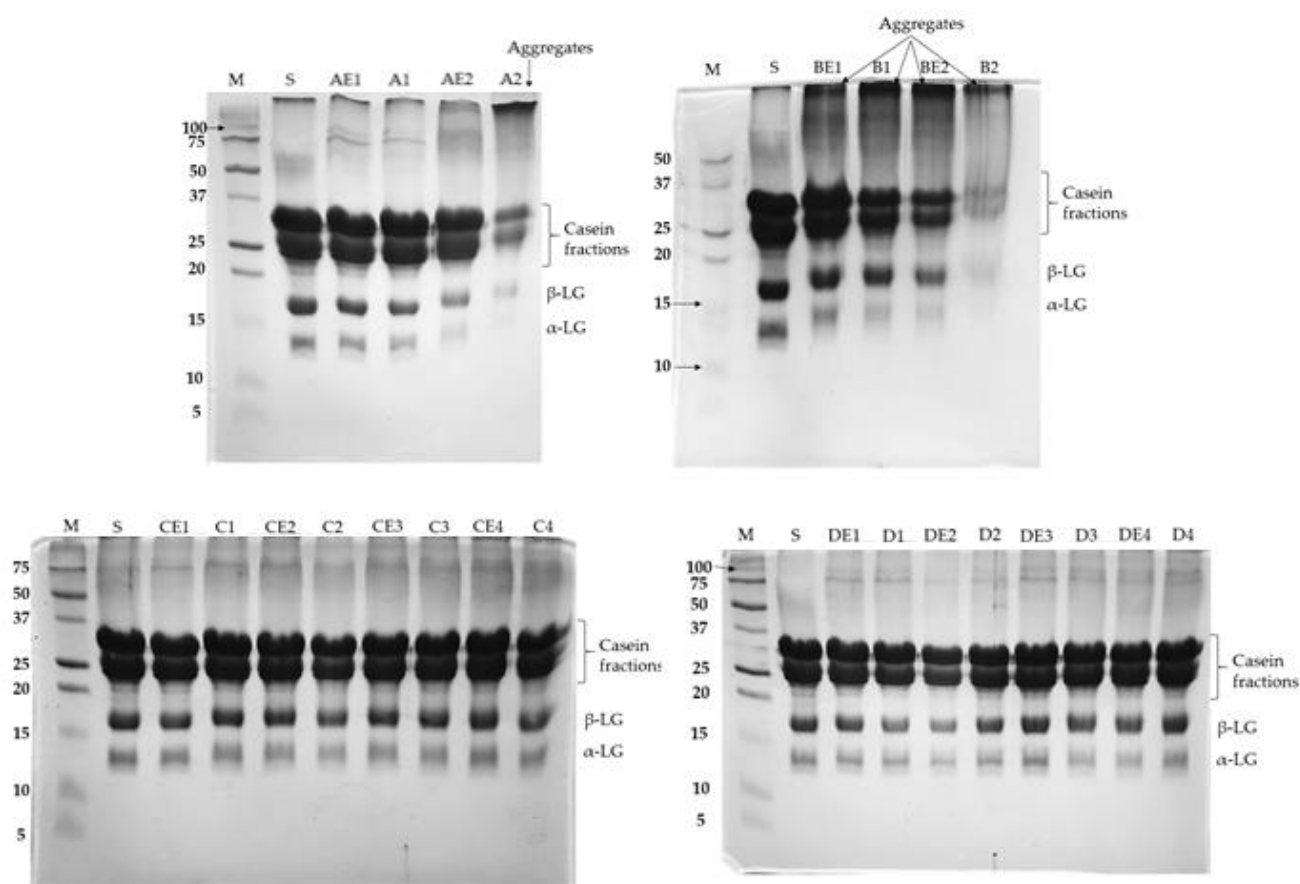
**Table 5.** Changes in indicators characterizing protein fraction of the sweetened condensed milk stored under various temperature conditions

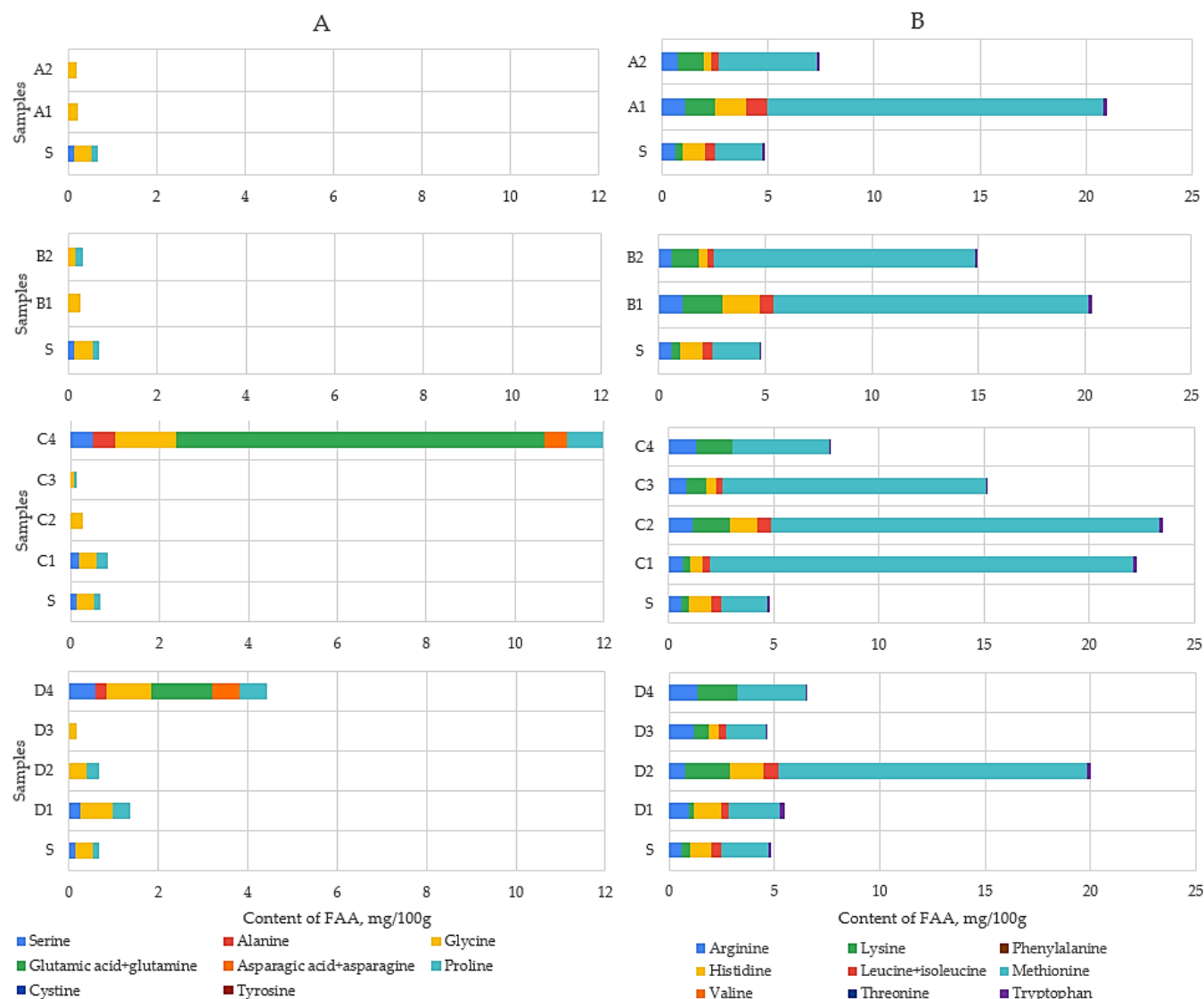
| Cycle type     | Indicators               |                           |                                                             |                          |                          |
|----------------|--------------------------|---------------------------|-------------------------------------------------------------|--------------------------|--------------------------|
| Name of sample | Total nitrogen, (%)      | Non-protein nitrogen, (%) | Real protein, (%) (TN <sup>†</sup> -NPN <sup>‡</sup> )·6.38 | Caseins, (%)             | Whey proteins, (%)       |
| S              | 1.279±0.019 <sup>a</sup> | 0.080±0.009 <sup>a</sup>  | 7.653±0.179 <sup>a</sup>                                    | 6.833±0.255 <sup>a</sup> | 0.820±0.012 <sup>a</sup> |
| S2             | 1.279±0.010 <sup>a</sup> | 0.096±0.008 <sup>a</sup>  | 7.544±0.115 <sup>a</sup>                                    | 6.725±0.172 <sup>a</sup> | 0.820±0.009 <sup>a</sup> |
| S3             | 1.264±0.036 <sup>a</sup> | 0.077±0.012 <sup>a</sup>  | 7.571±0.306 <sup>a</sup>                                    | 6.761±0.453 <sup>a</sup> | 0.810±0.023 <sup>a</sup> |
| AE1            | 1.296±0.009 <sup>a</sup> | 0.092±0.003 <sup>a</sup>  | 7.683±0.077 <sup>a</sup>                                    | 6.852±0.192 <sup>a</sup> | 0.831±0.018 <sup>a</sup> |
| A1             | 1.302±0.011 <sup>a</sup> | 0.097±0.009 <sup>a</sup>  | 7.685±0.128 <sup>a</sup>                                    | 6.850±0.293 <sup>a</sup> | 0.834±0.026 <sup>a</sup> |
| AE2            | 1.318±0.011 <sup>a</sup> | 0.096±0.008 <sup>a</sup>  | 7.793±0.121 <sup>a</sup>                                    | 6.948±0.319 <sup>a</sup> | 0.845±0.031 <sup>a</sup> |
| A2             | 1.259±0.041 <sup>a</sup> | 0.087±0.003 <sup>a</sup>  | 7.474±0.281 <sup>a</sup>                                    | 6.667±0.427 <sup>a</sup> | 0.807±0.023 <sup>a</sup> |
| BE1            | 1.299±0.006 <sup>a</sup> | 0.092±0.002 <sup>a</sup>  | 7.701±0.053 <sup>a</sup>                                    | 6.868±0.136 <sup>a</sup> | 0.833±0.013 <sup>a</sup> |
| B1             | 1.317±0.025 <sup>a</sup> | 0.094±0.002 <sup>a</sup>  | 7.803±0.170 <sup>a</sup>                                    | 6.958±0.183 <sup>a</sup> | 0.844±0.002 <sup>a</sup> |
| BE2            | 1.288±0.008 <sup>a</sup> | 0.093±0.002 <sup>a</sup>  | 7.623±0.065 <sup>a</sup>                                    | 6.797±0.096 <sup>a</sup> | 0.826±0.005 <sup>a</sup> |
| B2             | 1.259±0.042 <sup>a</sup> | 0.088±0.005 <sup>a</sup>  | 7.468±0.300 <sup>a</sup>                                    | 6.661±0.510 <sup>a</sup> | 0.807±0.033 <sup>a</sup> |
| C1             | 1.294±0.010 <sup>a</sup> | 0.096±0.009 <sup>a</sup>  | 7.642±0.121 <sup>a</sup>                                    | 6.812±0.179 <sup>a</sup> | 0.829±0.009 <sup>a</sup> |
| C2             | 1.292±0.009 <sup>a</sup> | 0.095±0.008 <sup>a</sup>  | 7.638±0.108 <sup>a</sup>                                    | 6.810±0.185 <sup>a</sup> | 0.828±0.012 <sup>a</sup> |
| C3             | 1.286±0.007 <sup>a</sup> | 0.094±0.001 <sup>a</sup>  | 7.602±0.051 <sup>a</sup>                                    | 6.778±0.204 <sup>a</sup> | 0.824±0.024 <sup>a</sup> |
| C4             | 1.262±0.038 <sup>a</sup> | 0.078±0.012 <sup>a</sup>  | 7.556±0.319 <sup>a</sup>                                    | 6.746±0.504 <sup>a</sup> | 0.809±0.029 <sup>a</sup> |
| D1             | 1.316±0.021 <sup>a</sup> | 0.088±0.006 <sup>a</sup>  | 7.831±0.172 <sup>a</sup>                                    | 6.988±0.230 <sup>a</sup> | 0.843±0.009 <sup>a</sup> |
| D2             | 1.284±0.010 <sup>a</sup> | 0.094±0.001 <sup>a</sup>  | 7.591±0.069 <sup>a</sup>                                    | 6.768±0.210 <sup>a</sup> | 0.823±0.022 <sup>a</sup> |
| D3             | 1.269±0.009 <sup>a</sup> | 0.092±0.001 <sup>a</sup>  | 7.508±0.064 <sup>a</sup>                                    | 6.694±0.281 <sup>a</sup> | 0.813±0.034 <sup>a</sup> |
| D4             | 1.264±0.030 <sup>a</sup> | 0.077±0.013 <sup>a</sup>  | 7.570±0.274 <sup>a</sup>                                    | 6.760±0.504 <sup>a</sup> | 0.810±0.036 <sup>a</sup> |

<sup>a</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA;  $p < 0.05$ .

<sup>†</sup> Total nitrogen,

<sup>‡</sup> Non-protein nitrogen

**Figure 2.** Electrophoregrams of the protein profiles of the samples.



**Figure 3.** Free amino-acid distributions in the samples of sweetened condensed milk stored under various temperature conditions. A, non-essential amino acids; and B, essential amino acids.

In the heating-cooling cycle of the multiple-stage storage cycle (Path C), destabilized fat content was detected after the cooling period to negative temperatures, which was seen in the subsequent samples until the end of the cycling experiment. Japanese researchers reported that the maximum temperature of ice formation was from 0 to  $-5^{\circ}\text{C}$  and freezing-thawing cycles carried out in this range, causing destruction of the physical structure of frozen products [23]. For the frozen SCM of this study, formation of destabilized fat was seen after the product passed lower temperatures ( $-50^{\circ}\text{C}$ ), which might indicate various temperatures of ice formation in SCM containing cryoprotectants (lactose, fats and proteins). The multiple-stage freezing-thawing cycle included storage of SCM in the range of maximal ice formation temperature for 96 h, compared to the single-stage cycle where time included only a few hours. Microscopic

analysis of the fat globules did not reveal changes in their average size from the storage temperature conditions. The average size of fat globules was within the range of  $2\text{--}3\text{ }\mu\text{m}$ , typical for SCM produced within an appropriate homogenization process, which was verified by stable values of the HD. Study of the FA composition of SCM did not reveal significant changes in the content of saturated, mono-unsaturated and polyunsaturated fatty acids (SFAs, MUFAs and PUFAs, respectively). The FA composition of all samples corresponded to the range of FA content typical for concentrated milks. Minor differences in the values of individual FAs were due to the effects of multiple-stage storage cycle and formation of free fatty acids (FFAs) in the process.

**Table 6.** Changes in indicators characterizing fat fraction of the sweetened condensed milk stored under various temperature conditions

| Cycle type     | Indicators                 |                       |                             |                             |
|----------------|----------------------------|-----------------------|-----------------------------|-----------------------------|
| Name of sample | Homogenization degree, (%) | Destabilized fat, (%) | Average globules size, (μm) | Maximum globules size, (μm) |
| S              | 41                         | abs.                  | 2.33±0.06 <sup>de</sup>     | 9.7±0.85 <sup>abc</sup>     |
| SE1            | 40                         | abs.                  | 2.10±0.14 <sup>e</sup>      | 10.00±0.94 <sup>abc</sup>   |
| S2             | 40                         | abs.                  | 2.37±0.06 <sup>de</sup>     | 8.34±1.04 <sup>abcdef</sup> |
| SE2            | 40                         | abs.                  | 2.27±0.11 <sup>e</sup>      | 9.00±0.91 <sup>abcde</sup>  |
| S3             | 38                         | abs.                  | 2.28±0.11 <sup>e</sup>      | 8.20±1.01 <sup>abcdef</sup> |
| AE1            | 41                         | abs.                  | 2.80±0.14 <sup>abc</sup>    | 10.90±0.95 <sup>a</sup>     |
| A1             | 39                         | abs.                  | 2.26±0.09 <sup>e</sup>      | 7.23±1.12 <sup>cdef</sup>   |
| AE2            | 40                         | abs.                  | 2.20±0.14 <sup>e</sup>      | 6.85±0.80 <sup>def</sup>    |
| A2             | 40                         | abs.                  | 2.65±0.11 <sup>abcd</sup>   | 6.69±0.70 <sup>def</sup>    |
| BE1            | 40                         | abs.                  | 2.64±0.12 <sup>bcd</sup>    | 7.46±0.61 <sup>bcddef</sup> |
| B1             | 40                         | abs.                  | 2.40±0.13 <sup>de</sup>     | 10.80±1.07 <sup>a</sup>     |
| BE2            | 40                         | abs.                  | 2.31±0.07 <sup>de</sup>     | 8.75±0.87 <sup>abcde</sup>  |
| B2             | 40                         | abs.                  | 2.10±0.14 <sup>e</sup>      | 6.61±0.71 <sup>def</sup>    |
| CE1            | 40                         | abs.                  | 2.64±0.13 <sup>bcd</sup>    | 7.44±0.74 <sup>bcddef</sup> |
| C1             | 40                         | abs.                  | 2.35±0.12 <sup>de</sup>     | 6.70±0.82 <sup>def</sup>    |
| CE2            | 41                         | abs.                  | 2.36±0.13 <sup>de</sup>     | 10.10±0.53 <sup>ab</sup>    |
| C2             | 40                         | trace                 | 2.27±0.11 <sup>e</sup>      | 8.86±1.12 <sup>abcde</sup>  |
| CE3            | 41                         | trace                 | 2.34±0.12 <sup>de</sup>     | 5.64±1.13 <sup>f</sup>      |
| C3             | 41                         | trace                 | 2.30±0.09 <sup>de</sup>     | 7.72±0.93 <sup>bcddef</sup> |
| CE4            | 40                         | trace                 | 2.41±0.11 <sup>de</sup>     | 8.59±0.80 <sup>abcde</sup>  |
| C4             | 40                         | trace                 | 2.37±0.10 <sup>de</sup>     | 8.12±0.79 <sup>abcdef</sup> |
| DE1            | 40                         | abs.                  | 2.77±0.06 <sup>abc</sup>    | 9.43±0.74 <sup>abcd</sup>   |
| D1             | 40                         | abs.                  | 2.20±0.14 <sup>e</sup>      | 7.30±0.89 <sup>bcddef</sup> |
| DE2            | 40                         | abs.                  | 2.11±0.12 <sup>e</sup>      | 6.37±0.71 <sup>ef</sup>     |
| D2             | 40                         | trace                 | 2.90±0.15 <sup>ab</sup>     | 9.84±0.95 <sup>abc</sup>    |
| DE3            | 41                         | trace                 | 2.64±0.11 <sup>bcd</sup>    | 6.40±0.91 <sup>ef</sup>     |
| D3             | 41                         | trace                 | 3.00±0.12 <sup>a</sup>      | 6.86±0.81 <sup>def</sup>    |
| DE4            | 40                         | trace                 | 2.24±0.03 <sup>e</sup>      | 8.25±0.97 <sup>abcdef</sup> |
| D4             | 38                         | trace                 | 2.45±0.07 <sup>cde</sup>    | 7.45±0.94 <sup>bcddef</sup> |

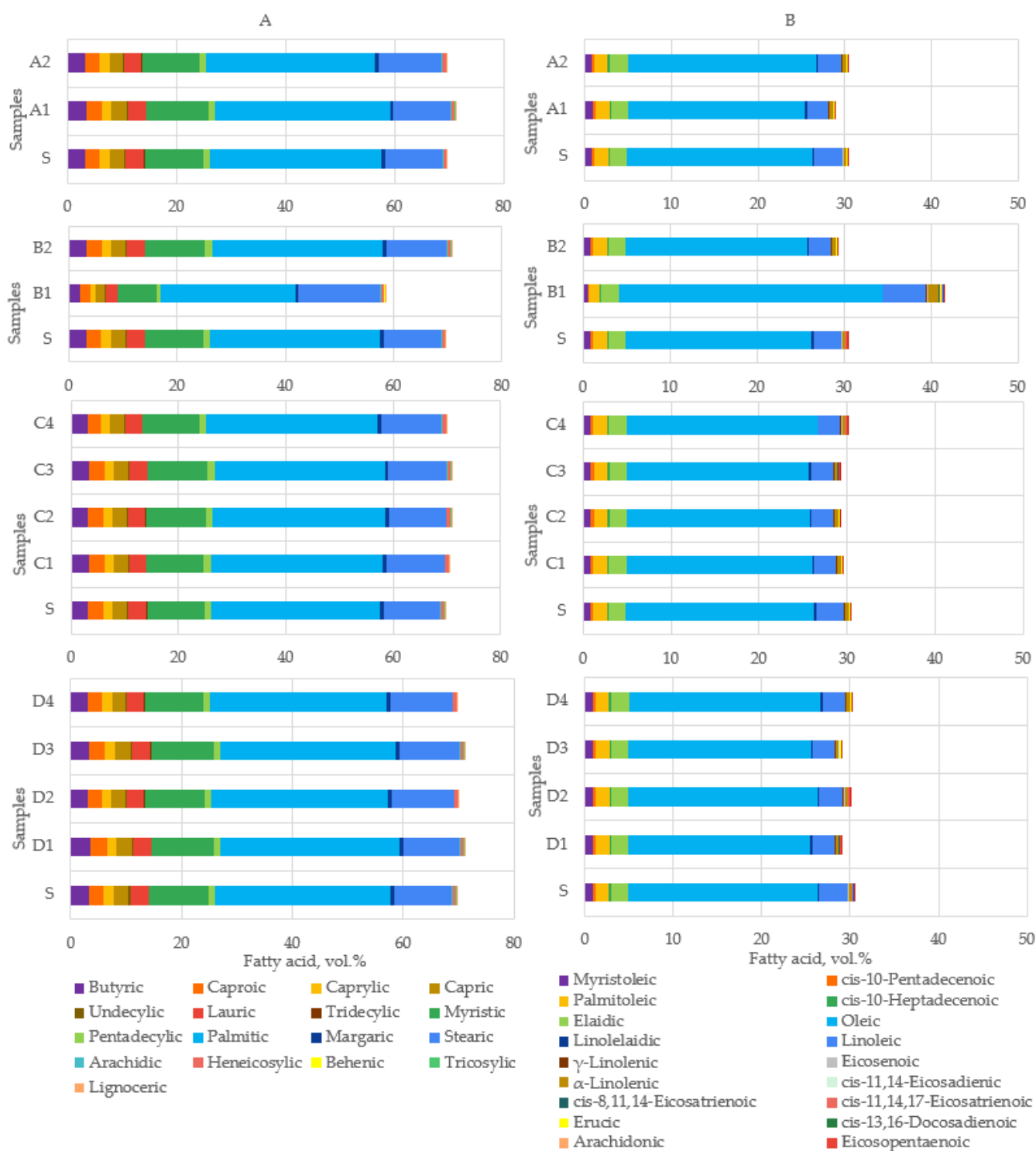
<sup>a-f</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA;  $p < 0.05$ .

### 3.5. Changes in the microbiological indicators of sweetened condensed milk

At long-term storage of SCM (standard conditions of temperature not higher than 10 °C and relative humidity not higher than 85%), viability of residual microbiota is gradually suppressed due to the osmotic pressure created by sucrose. However, transportation and storage of the product in unregulated (critical) temperature conditions can activate propagation of microorganisms and promote their propagation at  $A_w$  values much less than 0.83 with preservation of the ability to reproduce. In addition, species of microorganisms can produce intracellular lipolytic and proteolytic enzymes that affect constituents of the milk-sugar

system, thus causing undesirable defects (e.g., fat lipolysis and protein proteolysis). Hence, microbiological indicators (yeasts, molds and psychrotrophic, proteolytic and lipolytic microorganisms) were investigated (Table 7).

Data in Table 7 listed total viable count and total coliforms count, pathogens with the standard requirements within the storage modelling critical conditions. Count of lipolytic microorganism, yeast and mold did not exceed 10 CFU·g<sup>-1</sup> in all samples. Count of detected proteolytic micro-organisms did not exceed 100 CFU·g<sup>-1</sup>, including insignificant zigzag patterns in the process of temperature effects. Dependence of the effects of critical storage conditions (one-stage and multiple-stage heat exposures) on changes in the count of psychrotrophic microorganisms was reported.



**Figure 4.** Fatty acid compositions of the samples of sweetened condensed milk stored under various temperature conditions. A, saturated fatty acids; and B, unsaturated fatty acids.

At a single-stage temperature decrease of 100 °C (the shock effect) in the cycle from -50 to 50 °C and in the cycle from 50 to -50 °C, increases in the number of psychrotrophic microorganisms ( $7.5 \cdot 10^3$  and  $1.0 \cdot 10^3$  CFU·g<sup>-1</sup>) were recorded, following by their decreases to almost the initial values ( $< 10$  CFU·g<sup>-1</sup>) after cooling/heating to 5 °C. As a result of multiple-stage temperature exposure, increases in

the count of psychrotrophic microorganisms were seen regardless of the order of heating and freezing cycles, possibly linking to the propagation of their resting forms. However, microbial counts in the samples that were subjected to heating-to-freezing paths were slightly lower ( $8.9 \cdot 10^2$  CFU·g<sup>-1</sup>), compared to the samples that were subjected to freezing-to-heating paths ( $1.9 \cdot 10^3$  CFU·g<sup>-1</sup>).



Table 7. Changes in the microbiological indicators of sweetened condensed milk

| Cycle type     | Microorganism Groups                       |                                          |                                             |                                                             |                                                  |
|----------------|--------------------------------------------|------------------------------------------|---------------------------------------------|-------------------------------------------------------------|--------------------------------------------------|
| Name of sample | Total viable count, (CFU·g <sup>-1</sup> ) | Yeasts and molds, (CFU·g <sup>-1</sup> ) | Total psychrotrophic (CFU·g <sup>-1</sup> ) | Total bacteria,proteolytic bacteria, (CFU·g <sup>-1</sup> ) | Total lipolytic bacteria, (CFU·g <sup>-1</sup> ) |
| S              | (2.7±0.2)·10 <sup>3</sup> fgh              | <10 <sup>a</sup>                         | <10 <sup>e</sup>                            | (1.0±0.1)·10 <sup>1</sup> e                                 | <10                                              |
| SE1            | (3.7±0.3)·10 <sup>3</sup> fg               | (1.0±0.1)·10 <sup>1</sup> a              | <10 <sup>e</sup>                            | (1.0±0.1)·10 <sup>1</sup> e                                 | <10                                              |
| S2             | (4.4±0.2)·10 <sup>3</sup> efg              | <10 <sup>a</sup>                         | (5.6±0.3)·10 <sup>1</sup> e                 | (8.0±0.8)·10 <sup>1</sup> b                                 | <10                                              |
| SE2            | (5.0±0.5)·10 <sup>3</sup> ef               | <10 <sup>a</sup>                         | (7.0±0.6)·10 <sup>1</sup> e                 | (6.0±0.5)·10 <sup>1</sup> c                                 | <10                                              |
| S3             | (8.9±0.8)·10 <sup>3</sup> d                | <10 <sup>a</sup>                         | (2.9±0.3)·10 <sup>2</sup> e                 | (1.0±0.1)·10 <sup>2</sup> a                                 | <10                                              |
| A1             | (1.5±0.1)·10 <sup>2</sup> i                | <10 <sup>a</sup>                         | (1.0±0.1)·10 <sup>3</sup> d                 | (1.0±0.1)·10 <sup>1</sup> e                                 | <10                                              |
| A2             | (2.0±0.2)·10 <sup>3</sup> ghi              | <10 <sup>a</sup>                         | <10 <sup>e</sup>                            | (2.0±0.2)·10 <sup>1</sup> de                                | <10                                              |
| B1             | (2.0±0.2)·10 <sup>4</sup> a                | (1.0±0.1)·10 <sup>1</sup> a              | (7.5±0.6)·10 <sup>3</sup> a                 | (9.0±0.6)·10 <sup>1</sup> ab                                | <10                                              |
| B2             | (3.0±0.2)·10 <sup>2</sup> hi               | <10 <sup>a</sup>                         | <10 <sup>e</sup>                            | (3.0±0.2)·10 <sup>1</sup> d                                 | <10                                              |
| C1             | (6.5±0.6)·10 <sup>3</sup> de               | <10 <sup>a</sup>                         | <10 <sup>e</sup>                            | <10 <sup>e</sup>                                            | <10                                              |
| C2             | (1.0±0.1)·10 <sup>3</sup> hi               | (1.0±0.1)·10 <sup>1</sup> a              | (1.3±0.1)·10 <sup>3</sup> d                 | (1.0±0.1)·10 <sup>1</sup> e                                 | <10                                              |
| C3             | (2.0±0.1)·10 <sup>3</sup> ghi              | <10 <sup>a</sup>                         | (1.0±0.1)·10 <sup>2</sup> e                 | <10 <sup>e</sup>                                            | <10                                              |
| C4             | (4.0±0.2)·10 <sup>3</sup> fg               | <10 <sup>a</sup>                         | (8.9±0.5)·10 <sup>2</sup> d                 | <10 <sup>e</sup>                                            | <10                                              |
| D1             | (2.6±0.3)·10 <sup>2</sup> i                | <10 <sup>a</sup>                         | <10 <sup>e</sup>                            | (1.0±0.1)·10 <sup>1</sup> e                                 | <10                                              |
| D2             | (1.2±0.1)·10 <sup>4</sup> c                | <10 <sup>a</sup>                         | (6.2±0.5)·10 <sup>3</sup> b                 | (9.0±0.7)·10 <sup>1</sup> ab                                | <10                                              |
| D3             | (1.5±0.2)·10 <sup>4</sup> b                | <10 <sup>a</sup>                         | (2.0±0.1)·10 <sup>1</sup> e                 | <10 <sup>e</sup>                                            | <10                                              |
| D4             | (3.5±0.2)·10 <sup>3</sup> fg               | <10 <sup>a</sup>                         | (1.9±0.1)·10 <sup>3</sup> c                 | <10 <sup>e</sup>                                            | <10                                              |

<sup>a-h</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA; *p*< 0.05.

These results on changes of total psychrotrophic bacteria during freezing and thawing and heating and cooling processes are similar to those by McKellar [24] and Mahjoubin-Tehran et al. [25], who highlighted that storage temperature of milk and milk products at temperatures under 7 °C could stimulate psychrotrophic microorganisms.

3.6. Changes of sensory indicators of sweetened condensed milk

Another aim of this study was to investigate sensory indicators of the samples (Fig. 5).

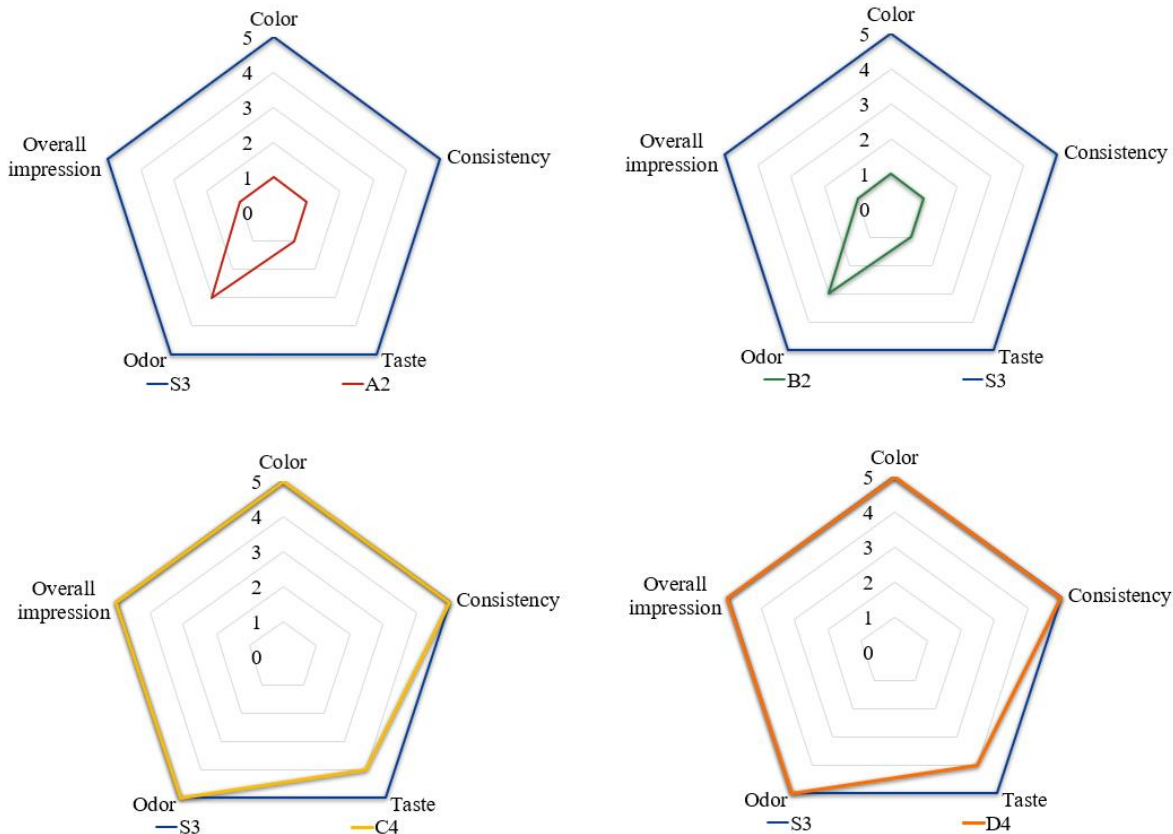


Figure 5. Changes in sensory indicators of the sweetened condensed milk

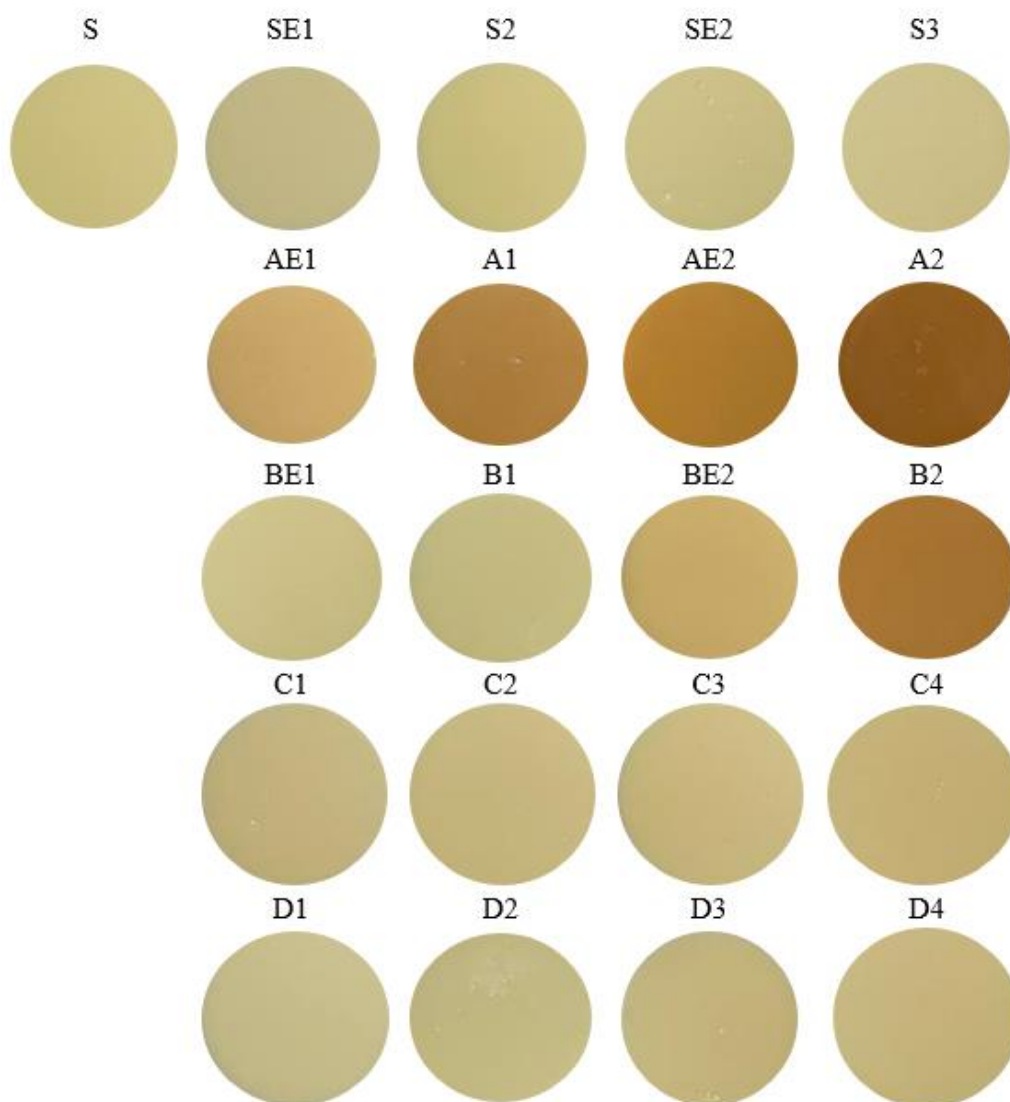
Samples of Paths A and B, which at the end of the cyclic experiment were classified as defective, included significant changes. They were characterized by dark brown color and caramel taste typical for boiled SCM, which were connected to increases in acidity, browning index and viscosity. Furthermore, Path A samples were characterized by a sour taste, which was detected in Path B and was stronger than that in Path A. Path C and D samples were subjected to little changes at the end of the experiment and scored lower only on the taste indicator, which was characterized by slight bitterness and stronger sweetness, compared to the control samples. This might be linked to the sucrose hydrolysis, a possible reason of the freezing point decreases.

Samples of Path B included decreased (by 0.5 point) scores on the taste indicator only after a period of cooling-freezing and heating to 5 °C, which was characterized by a mild perceptible taste of bitterness. Control samples fully

met the standards for sensory indicators. In D4 sample, a mild color change (light creamy tinge) was recorded that was allowed by the standards, as well as a strong sweet taste. Sensory profile of the B2 sample after heat exposure corresponded to the characteristics typical for boiled condensed milks as color of the sample became brown, viscosity and acidity increased and taste was dominated by the flavor of baked milk, which was associated with the intense melanoidin formation reaction. Exemplification of the appearance of the samples is present in Fig. 6.

### 3.7. Quality changes of the sweetened condensed milk during storage

The SCM in storage Paths A, B, C and D was stored and its quality indicators and changes in characteristics characterizing the transformation of product determinants (fats, proteins and carbohydrates) were analyzed (Table 8).



**Figure 6.** Visualization of the color changes in the sweetened condensed milk samples during storage.

**Table 8.** Changes in SCM indicators during 6 m of storage

| Name of parameter          | Period of storage   |                     |                     |                     |                        |                     |                        |                      |                      |                        |                       |                     |                       |                       |                     |                      |
|----------------------------|---------------------|---------------------|---------------------|---------------------|------------------------|---------------------|------------------------|----------------------|----------------------|------------------------|-----------------------|---------------------|-----------------------|-----------------------|---------------------|----------------------|
|                            | 0 month             |                     | 1 month             |                     |                        |                     | 3 month                |                      |                      |                        |                       | 6 month             |                       |                       |                     |                      |
|                            | S                   | S3                  | A2                  | B2                  | C4                     | D4                  | S5                     | A4                   | B4                   | C6                     | D6                    | S8                  | A7                    | B7                    | C9                  | D9                   |
| Water activity (/10)       | 8.48±               | 8.49±               | 8.53±               | 8.50±               | 8.49±                  | 8.49±               | 8.43±                  | 8.46±                | 8.43±                | 8.44±                  | 8.45±                 | 8.56±               | 8.53±                 | 8.57±                 | 8.57±               | 8.56±                |
|                            | 0.02 <sup>ab</sup>  | 0.03 <sup>ab</sup>  | 0.03 <sup>a</sup>   | 0.04 <sup>ab</sup>  | 0.03 <sup>ab</sup>     | 0.01 <sup>ab</sup>  | 0.02 <sup>b</sup>      | 0.01 <sup>a</sup>    | 0.02 <sup>ab</sup>   | 0.03 <sup>ab</sup>     | 0.04 <sup>ab</sup>    | 0.03 <sup>ab</sup>  | 0.04 <sup>a</sup>     | 0.03 <sup>ab</sup>    | 0.03 <sup>ab</sup>  | 0.04 <sup>ab</sup>   |
| Titratable acidity, °T     | 41±2 <sup>d</sup>   | 40±3 <sup>d</sup>   | 69±2 <sup>a</sup>   | 64±2 <sup>ab</sup>  | 41±2 <sup>d</sup>      | 42±1 <sup>d</sup>   | 43±1 <sup>d</sup>      | 64±3 <sup>ab</sup>   | 60±2 <sup>bc</sup>   | 44±2 <sup>d</sup>      | 43±1 <sup>d</sup>     | 41±1 <sup>d</sup>   | 62±2 <sup>bc</sup>    | 58±1 <sup>c</sup>     | 42±1 <sup>d</sup>   | 42±1 <sup>d</sup>    |
|                            | 6.36±               | 6.40±               | 5.99±               | 5.93±               | 6.35±                  | 6.36±               | 6.42±                  | 5.99±                | 6.03±                | 6.36±                  | 6.34±                 | 6.50±               | 5.97±                 | 6.05±                 | 6.42±               | 6.43±                |
| pH                         | 0.03 <sup>a</sup>   | 0.03 <sup>a</sup>   | 0.07 <sup>b</sup>   | 0.09 <sup>bc</sup>  | 0.04 <sup>a</sup>      | 0.03 <sup>a</sup>   | 0.03 <sup>a</sup>      | 0.08 <sup>b</sup>    | 0.06 <sup>b</sup>    | 0.05 <sup>a</sup>      | 0.04 <sup>a</sup>     | 0.04 <sup>a</sup>   | 0.07 <sup>b</sup>     | 0.09 <sup>b</sup>     | 0.04 <sup>a</sup>   | 0.03 <sup>a</sup>    |
|                            | 8.4±                | 12.0±               | 53.4±               | 53.1±               | 14.7±                  | 19.7±               | 11.3±                  | 47.2±                | 37.4±                | 21.2±                  | 21.8±                 | 12.1±               | 39.1±                 | 33.4±                 | 19.2±               | 20.2±                |
| Geppler Viscosity, Pa·s    | 0.2 <sup>k</sup>    | 0.4 <sup>j</sup>    | 0.5 <sup>a</sup>    | 0.7 <sup>a</sup>    | 0.2 <sup>i</sup>       | 0.8 <sup>gh</sup>   | 0.3 <sup>j</sup>       | 0.2 <sup>b</sup>     | 0.4 <sup>d</sup>     | 0.6 <sup>fg</sup>      | 0.8 <sup>f</sup>      | 0.5 <sup>j</sup>    | 0.4 <sup>c</sup>      | 0.7 <sup>e</sup>      | 0.6 <sup>h</sup>    | 0.4 <sup>gh</sup>    |
|                            | 8.9±                | 13.9±               | >64 <sup>a</sup>    | >64 <sup>a</sup>    | 22.2±                  | 22.9±               | 14.6±                  | >64 <sup>a</sup>     | >64 <sup>a</sup>     | 25.2±                  | 25.2±                 | 16.7±               | >64 <sup>a</sup>      | >64 <sup>a</sup>      | 23.6±               | 26±                  |
| Brookfield Viscosity, Pa·s | 1.1 <sup>d</sup>    | 1.0 <sup>c</sup>    |                     |                     | 1.9 <sup>b</sup>       | 1.6 <sup>b</sup>    | 1.2 <sup>c</sup>       |                      |                      | 2.4 <sup>b</sup>       | 1.7 <sup>b</sup>      | 1.5 <sup>c</sup>    |                       |                       | 2.2 <sup>b</sup>    | 1.5 <sup>b</sup>     |
|                            | 12.317±             | 12.300±             | 12.250±             | 11.794±             | 12.308±                | 12.415±             | 12.395±                | 12.298±              | 10.547±              | 11.891±                | 12.236±               | 12.237±             | 12.289±               | 10.536±               | 11.989±             | 12.112±              |
| Lactose, %                 | 0.081 <sup>ab</sup> | 0.085 <sup>ab</sup> | 0.144 <sup>ab</sup> | 0.134 <sup>bc</sup> | 0.062 <sup>a</sup>     | 0.073 <sup>ab</sup> | 0.112 <sup>ab</sup>    | 0.111 <sup>ab</sup>  | 0.113 <sup>c</sup>   | 0.104 <sup>bc</sup>    | 0.138 <sup>ab</sup>   | 0.071 <sup>ab</sup> | 0.065 <sup>ab</sup>   | 0.132 <sup>c</sup>    | 0.075 <sup>ab</sup> | 0.105 <sup>ab</sup>  |
|                            | -0.415±             | -0.411±             | -0.439±             | -0.432±             | -0.405±                | -0.431±             | -0.402±                | -0.401±              | -0.407±              | -0.403±                | -0.406±               | -0.392±             | -0.398±               | -0.398±               | -0.394±             | -0.395±              |
| Freezing point, °C         | 0.003 <sup>b</sup>  | 0.004 <sup>bc</sup> | 0.002 <sup>a</sup>  | 0.004 <sup>a</sup>  | 0.004 <sup>bcdef</sup> | 0.005 <sup>a</sup>  | 0.005 <sup>cdefg</sup> | 0.001 <sup>bcd</sup> | 0.002 <sup>bcd</sup> | 0.002 <sup>cdefg</sup> | 0.004 <sup>bcde</sup> | 0.004 <sup>g</sup>  | 0.004 <sup>defg</sup> | 0.004 <sup>defg</sup> | 0.004 <sup>fg</sup> | 0.004 <sup>efg</sup> |
|                            | 1.279±              | 1.264±              | 1.259±              | 1.259±              | 1.262±                 | 1.264±              | 1.252±                 | 1.284±               | 1.250±               | 1.257±                 | 1.259±                | 1.261±              | 1.247±                | 1.259±                | 1.259±              | 1.256±               |
| Total nitrogen, %          | 0.019 <sup>a</sup>  | 0.036 <sup>a</sup>  | 0.041 <sup>a</sup>  | 0.042 <sup>a</sup>  | 0.038 <sup>a</sup>     | 0.030 <sup>a</sup>  | 0.018                  | 0.024                | 0.033                | 0.023                  | 0.015                 | 0.017               | 0.038                 | 0.024                 | 0.040               | 0.031                |
|                            | 0.080±              | 0.077±              | 0.087±              | 0.088±              | 0.078±                 | 0.077±              | 0.080±                 | 0.083±               | 0.083±               | 0.077±                 | 0.077±                | 0.078±              | 0.086±                | 0.085±                | 0.081±              | 0.080±               |
| Non-protein nitrogen, %    | 0.009 <sup>a</sup>  | 0.012 <sup>a</sup>  | 0.003 <sup>a</sup>  | 0.005 <sup>a</sup>  | 0.012 <sup>a</sup>     | 0.013 <sup>a</sup>  | 0.008 <sup>a</sup>     | 0.008 <sup>a</sup>   | 0.012 <sup>a</sup>   | 0.002 <sup>a</sup>     | 0.014 <sup>a</sup>    | 0.010 <sup>a</sup>  | 0.011 <sup>a</sup>    | 0.012 <sup>a</sup>    | 0.006 <sup>a</sup>  | 0.012 <sup>a</sup>   |
|                            | 46                  | 38                  | 40                  | 40                  | 40                     | 42                  | 38                     | 40                   | 40                   | 40                     | 43                    | 38                  | 40                    | 40                    | 40                  | 40                   |
| Homogenization degree, %   |                     |                     |                     |                     |                        |                     |                        |                      |                      |                        |                       |                     |                       |                       |                     |                      |
| Destabilized fat, %        | -                   | -                   | -                   | -                   | trace                  | -                   | trace                  | -                    | -                    | trace                  | trace                 | trace               | -                     | -                     | -                   | trace                |

<sup>a-g</sup> Means followed by different lowercase letters in rows differ significantly by ANOVA;  $p < 0.05$ .

In control samples and samples of storage Paths C and D during 6 m, acidity values did not change significantly and corresponded to the standard GOST 31688-2012 (not greater than 48 °T). In samples of Paths A and B, these did not exceed the standard limits. Changes in pH correlated with the changes of titratable acidity. In storage after cycles of the thermal exposure of various types, thickening was reported in all the samples, except for the control one. It was reported that critical thermal effects activated the thickening process, which was possibly associated with the thermal stimulation of changes in the protein composition. Relatively, [26] presented data that slow irreversible changes might occur in the size or shape of casein micelles in the process of gelation, which led to increases in the constant viscosity. To stimulate the thickening of SCM and study its rheological characteristics, researchers of this study used a storage mode at 39 °C for 3 w. Viscosity (by Heppler), which was an indicator depending on changes of acidity and indirectly indicating changes of the protein fraction, insignificantly changed in the control samples from Months 1 to 6. For the samples of Paths C and D, viscosities were not statistically different after 3 and 6 m. Values for paths increased by Month 3 (44 and 11%, respectively) and decreased mildly by Month 6 (9 and 7%, respectively). Viscosities of Paths A and B samples decreased from Months 1 to 6 of storage by 26 and 37%, respectively. After 1 m of storage, viscosity values for Paths A and B were not statistically different. It is noteworthy that Brookfield viscosity measurement showed no significant differences in viscosity values for Paths C and D from Months 1 to 6. Viscosities of these samples were not statistically significant. However, Brookfield viscosity did not allow comparisons of the two types of the thermal effects of A and B cycles, since viscosity values for this method were greater than 64 Pa·s as the measurement limit of the instrument.

In all samples, changes in  $A_w$ , HD, TN and NPN were not statistically significant, indicating that thermal effects of the types of A, B, C and D cycles did not include significant effects on changes in the osmotic pressure of the product, stability of the fat phase and content of the major indicators of protein composition. Content of lactose in the samples of Paths S, A, C and D from the beginning of storage to 6 m almost did not change and values for the samples of these paths were not statistically different from each other. Further significant changes in lactose content were reported for the samples of Path B in storage. From Months 1 to 3, values of the lactose content in samples of the path decreased by 10.6% and in Month 6, these values were statistically similar to

those in Month 3. This might be associated to the hydrolysis of lactose and monosaccharide interactions with the proteins. According to [16], reactions between lactose and  $\alpha$  and  $\epsilon$ -amino groups in milk proteins occur not only during heating but also continue during storage even at or under ambient temperatures. Increases in freezing point of all

samples in Month 6 of storage was most likely due to the formation of protein-carbohydrate compounds; for example, monosaccharides formed at the initial stage of storage (1 m) could further engage in the Maillard reaction, causing decreases in osmolality.

## 4. Conclusion

Analysis of the samples subjected to a cycle, including multiple-stage heating to 50 °C for 9 d followed by multiple-stage cooling to 5 °C for 11 d, revealed that only viscosity changed, relative to the control samples. In reverse similar cycles (cooling → heating), destabilized fats were seen. Additionally, it was revealed that changes of the cycles and subsequent storage of these samples for 6 m were characterized by the increased viscosity alone, compared with the control samples. It is possible to develop novel standards for SCM transported in uncontrolled temperature conditions (with gradual increases or decreases in temperature); in which, novel permissible viscosity ranges are introduced. The reported propagations of psychrotrophic and proteolytic microorganisms were not reported as specific characteristics of specific types of heat exposure since the control path showed changes in their counts of zigzag characters. Single-stage freezing with storage of the product within 14 d of SCM did not critically affect changes in the product quality. Rapid heating of SCM up to 50 °C and storing it in such critical conditions outside the refrigerated storage space are not admitted. Further storage results of the samples subjected to cycles of one-stage freezing and heating for 6 m showed complete insimilarities with those of the control samples for all the parameters. For sensory characteristics, these samples produced results similar to those that the baked SCM did. In breaking the storage protocols, it is possible to address such a SCM as a baked SCM product, providing that this product can meet the established standards. Results make it possible to increase limit values of the ambient temperature; at which, SCM can be transported to regions with critical temperature conditions (e.g., Asian and African countries and the Arctic). This novelty can expand the possibilities of SCM producers for export and sale, meaning increases in supply levels of consumers with highly-nutritious quality dairy products. Allowing transportation of SCM in uncontrolled temperature conditions at a range of -50 to 50 °C with increases in temperature per day by no more than 5 °C may allow SCM producers to decrease costs for the use of specialized transportation systems.

## 5. Acknowledgements

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assistance in the research and the manufacturer "Promkonservy" for providing condensed milk samples.

## 6. Conflict of Interest

The authors report no conflict of interest.

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# اثرات درجه حرارت‌های بحرانی نگهداری بر شاخص‌های میکروبی، فیزیکی-شیمیایی و حسی شیر تغلیظ شده شیرین

الکساندر کروچنین<sup>\*</sup>، النا یوروا، اکاترینا بولشاکووا، سوتلانا توروفسکایا، النا ایلاریونوا، ایرینا بارکوفسکایا، ویکتوریا لئونوا

موسسه تحقیقات لبنیات سراسر روسیه، لوسینوفسکایا ۷-۳۵، ۱۱۵۰۹۳ مسکو، روسیه

## چکیده

**سابقه و هدف:** برای تولید شیرهای تغلیظ شده شیرین از اصول اسمو و ترموآنابیز استفاده می‌شود. با توجه به طولانی شدن عمر انباری آنها، تقاضا برای صادرات آنها به کشورهای با آب و هوای گوناگون وجود دارد. با این حال، درجه حرارت‌های بسیار بالاتر و پایین‌تر از دمای محیط در طول حمل و نقل شیرهای تغلیظ شده شیرین می‌تواند بر کیفیت آنها تأثیر بگذارد. از این رو، مطالعه اثرات درجه حرارت‌های بحرانی نگهداری بر شاخص‌های میکروبی، فیزیکی-شیمیایی و حسی شیرهای تغلیظ شده شیرین بسیار مهم است.

**مواد و روش‌ها:** این تحقیق مطالعه جامعی از ویژگی‌های فیزیکی-شیمیایی، میکروبی و حسی شیرهای تغلیظ شده شیرین پس از نگهداری در شرایطی بود که شامل تغییرات دمایی چند مرحله‌ای و تک مرحله‌ای در محدوده‌های مختلف (۵ تا ۵۰ درجه سانتی‌گراد؛ از ۵ تا ۵۰- درجه سانتی‌گراد، ۵۰ تا ۵۰- درجه سانتی‌گراد و برعکس بود.

**یافته‌ها و نتیجه‌گیری:** آنالیز نمونه‌های قرار داده شده تحت تغییرات چرخه‌ای، از جمله گرمایش چند مرحله‌ای به مدت ۹ روز و سپس خنک‌سازی چند مرحله‌ای برای ۱۱ روز، نشان داد که تنها گران‌روی<sup>۱</sup> نسبت به نمونه‌های شاهد تغییر کرده است. در چرخه مشابه ولی برعکس (سرد کردن به گرمایش)، تشکیل چربی ناپایدار مشاهده شد. علاوه بر این، تغییر چرخه‌ها و پس از آن نگهداری نمونه‌ها ۶ ماه منجر به افزایش گران‌روی نسبت به نمونه‌های شاهد شد. مشخص شد که انجماد تک مرحله‌ای با ذخیره‌سازی ۱۴ روزه بر کیفیت آن تأثیری ندارد. در مقابل، حرارت دادن سریع شیر تغلیظ شده شیرین شده تا دمای ۵۰- درجه سانتی‌گراد و نگهداری در چنین شرایط بحرانی در خارج از یک منطقه ذخیره‌سازی سرد غیرقابل قبول بود. ذخیره‌سازی بعدی نمونه‌هایی که در معرض چرخه‌های انجماد و گرمایش تک مرحله‌ای به مدت ۶ ماه قرار گرفتند، عدم انطباق کامل با نمونه‌های شاهد را برای همه شاخص‌ها نشان داد. بنابراین، شیر تغلیظ شده شیرین را می‌توان در یک مرحله انجماد تا ۵۰- درجه سانتی‌گراد منجمد و به مدت ۱۴ روز نگهداری کرد، همچنین با سرد کردن/انجماد چند مرحله‌ای تا ۵۰- درجه سانتی‌گراد و حرارت دادن چند مرحله‌ای تا ۵۰- درجه سانتی‌گراد و سپس سرد کردن تا ۵- درجه سانتی‌گراد و بدون از دست دادن کیفیت و ایمنی در مدت ۶ ماه قابل نگهداری است.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافع مرتبط با انتشار این مقاله ندارند.

## تاریخچه مقاله

دریافت ۲۳ ژانویه ۲۰۲۴

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## واژگان کلیدی

- شیر تخمیر شده
- درجه حرارت‌های بحرانی نگهداری
- آنزیم‌های خارج سلولی
- تجزیه چربی و پروتئین
- باکتری‌های سرما دوست<sup>۲</sup>
- شیرهای تغلیظ شده شیرین
- حمل و نقل

## \*نویسنده مسئول

الکساندر کروچنین

موسسه تحقیقات لبنیات سراسر

روسیه، لوسینوفسکایا ۷۱۳۵،

۱۱۵۰۹۳ مسکو، روسیه

تلفن: ۷(۴۹۹)۲۳۶-۰۲-۳۶

پست الکترونیک:

[a.kruchinin@vniimi.org](mailto:a.kruchinin@vniimi.org)

<sup>۱</sup> viscosity

<sup>۲</sup> Psychotropic bacteria باکتری‌هایی که می‌توانند در دمای پایین، بین صفر تا پنج درجه سلسیوس، رشد کنند، هرچند دمای بهینه رشد آنها بین ۱۵ تا ۲۰ درجه سلسیوس است